Contemporary Diabetes
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The Diabetic Foot

Medical and Surgical Management

Fourth Edition

Humana Press

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Preface to the Fourth Edition

We are proud to present the fourth edition of our standard textbook *The Diabetic Foot* 5 years after its previous version. As in prior editions, we have tried to highlight new developments in our understanding of diabetic foot physiology and its clinical management. In order to best achieve our aim, we have divided the book into four sections, the first focusing on clinical features and diagnosis, the second on pathophysiology, and the third on the management of diabetic foot problems and the fourth on organization and preventive care. In addition to updating prior chapters, we have added several new contributions that reflect advances in our understanding of the causes of diabetic foot ulcers and efforts to develop new and more effective therapies.

In the 5 years since the last edition, it has become even more evident that the diabetes pandemic continues unabated, with millions of additional cases diagnosed each year. There is therefore no doubt that intense efforts by health care professionals and provider organizations throughout the world to develop clinical programs that can provide efficient and affordable diabetic foot care will be required. To this end, we hope our efforts in this book will help provide the necessary basics as we include fundamental principles for managing diabetic foot problems that have developed over five decades at the Joslin-Beth Israel Deaconess Diabetic Foot Center, one of the very first centers to focus on this condition in a systematic and multidisciplinary fashion.

As time goes by, changes in our editorial leadership occur as a matter of course. The replacement of Dr. Frank W. LoGerfo, co-editor in all three previous editions, by Dr. Raul J. Guzman in this edition is one such change. Dr. LoGerfo has recently retired from clinical practice and as such felt that it would be best to step down as co-editor and pass the baton to Dr. Guzman. First, we would like to thank Dr. LoGerfo for all of his significant contributions to this project over the years. We also want to recognize his noteworthy accomplishments related to care of the diabetic foot ulcer patient as it is common knowledge that without his early pioneering work, the surgical management of these patients would not have attained its current level of success. Finally, we would like to wish him every success in his future research and nonacademic endeavors. We also want to welcome Dr. Guzman, a vascular surgeon with extensive experience in managing diabetic foot ulcer patients and research interests in the pathophysiology of pedal ischemia, as our new co-editor for this edition.

We believe that the fact that we are publishing the fourth edition since the first was published in 2002 speaks loudly to the success of the previous efforts. We hope that the current edition will be equally successful and that it will help our diabetic foot patients receive better care and see tangible results in their fight to preserve an intact and functional lower extremity. As with the previous edition, we want to sincerely thank all the authors for their hard work in providing outstanding chapters. We also want to express our gratitude to Humana Press for their continuing support of this project.

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Part I

Clinical Features and Diagnosis

Epidemiology and Health Care Cost of Diabetic Foot Problems

1

Robert G. Frykberg, Jeremy J. Cook, and Donald C. Simonson

Abstract

The diabetic lower extremity has long been a cause for both morbidity and mortality in patients afflicted with this multisystem disease. Unfortunately, the global prevalence of diabetes mellitus has been projected to nearly double from a baseline of 2.8% in 2000 to 4.4% by 2030, affecting over 350 million individuals (Wild et al. Diabetes Care. 2004;27(5):1047–53). In the decade beginning in 1997, the prevalence of diabetes in the USA has increased by 48% (http://apps.nccd.cdc.gov/DDTSTRS/default.aspx). Lower extremity morbidity contributes substantially to the toll diabetes takes on the individual and the health care system. This chapter focuses on the epidemiologic aspects of risk factors and complications in the diabetic lower extremity, particularly as they relate to the outcome of amputation. Included in the discussion is the influence of demographic factors, such as gender, age, race, and socioeconomic considerations, as well as the cost to the health care system of lower extremity disease in diabetes.

Introduction

In his landmark paper of 1934, Eliott P. Joslin lamented on the "Menace of Diabetic Gangrene" and how its frequency was increasing among his patients [1]. With his keen insights and clinical acumen he was able to ascertain, even in the early twentieth century, those risk factors that placed the diabetic lower extremity at risk for ulceration, gangrene, and amputation. Many years later in 1992, Zimmet first referred to the "epidemic of diabetes," noting that its costs both in terms of economic burden and human suffering are rising at an alarming rate [2]. The global prevalence of diabetes mellitus has been projected to nearly double from a baseline of 2.8% in 2000 to 4.4% by 2030, affecting over 350 million individuals [3]. In the decade beginning in 1997, the prevalence of diabetes in the USA has increased by 48% [4] (Fig. 1.1). An estimated 29 million or 9.3% of people living in the USA are affected by diabetes mellitus, with its prevalence and costs continuing to increase [5]. In the years 2007 to 2013 the prevalence of diabetes increased by 26% with associated costs of this disease increasing by 41% [6, 7]. The total estimated cost of diabetes in 2012 was $245 billion, with 43% of costs attributed to inpatient care. Compared to people without diabetes, the medical expenditures are approximately 2.3-fold higher for diabetic persons [7]. Lower extremity morbidity contributes substantially to the toll diabetes takes on the individual and the health care system. In fact, of the 785 million ambulatory diabetes-related outpatient visits between 2007 and 2013, approximately 6.7 million visits (0.8%) were for diabetic foot ulcers (DFU) or infections. DFU visits were associated with a 3.4 greater odds of direct Emergency department or inpatient admission [8]. This chapter focuses on the epidemiologic aspects of risk factors and complications in the diabetic lower extremity, particularly as they relate to the outcome of amputation. Included in the discussion is the influence of demographic factors, such as gender, age, race, and socioeconomic considerations, as well as the cost to the health care system of lower extremity disease (LED) in diabetes.

Epidemiology of Individual Risk Factors

The individual systems at risk that predispose an individual to ulceration are covered in greater detail throughout this

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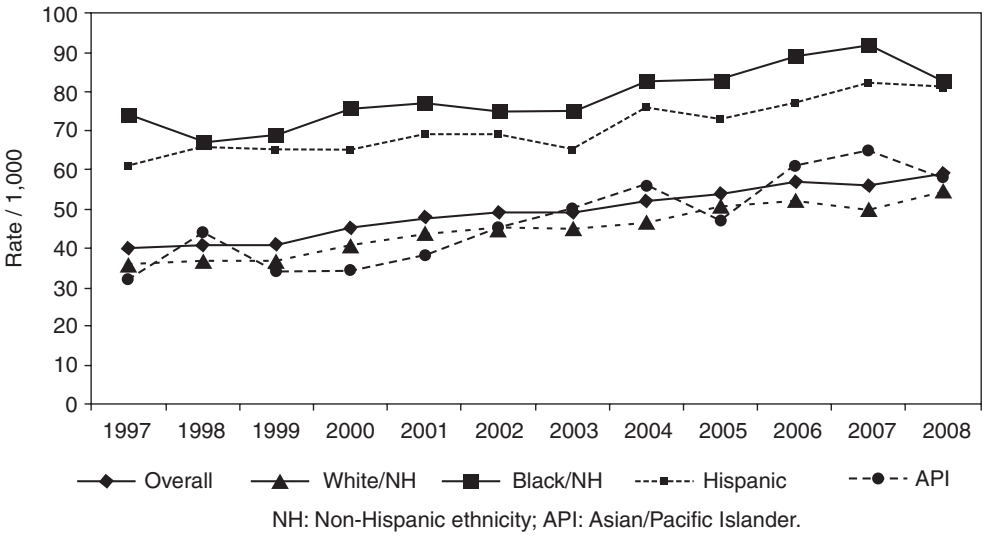


Fig. 1.1 Diabetes prevalence [4]

textbook. In this chapter, a brief introduction to these risk factors is presented as they relate to the epidemiology of the at-risk foot.

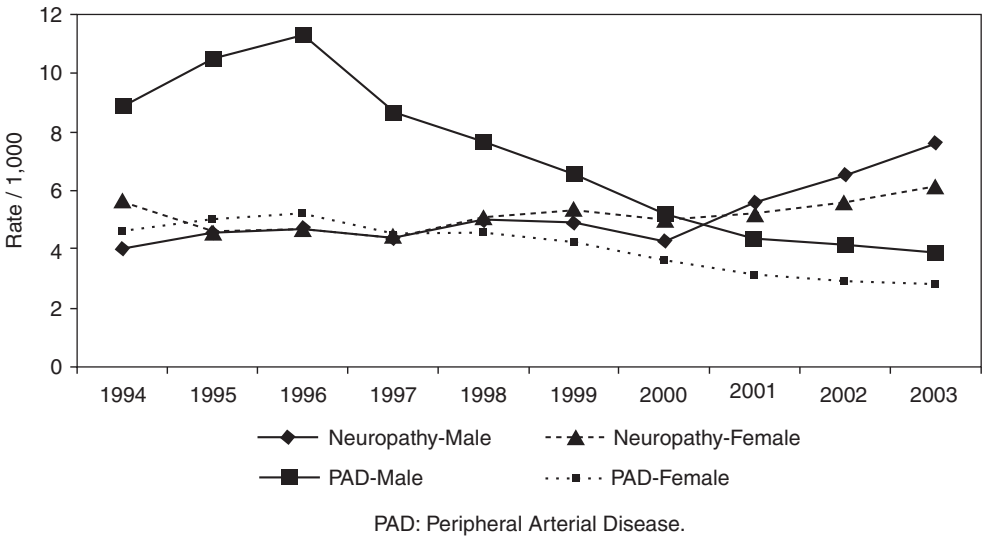
Neuropathy

A frequently encountered complication of diabetes mellitus is neuropathy. Diabetic peripheral neuropathy (DPN) is an impairment of normal activities of the nerves throughout the body and can alter autonomic, motor, and sensory functions [9]. The reported prevalence of DPN ranges from 16% to as high as 66% [10–14]. According to a study utilizing National Health and Nutrition Examination Survey (NHANES) data of 2873 noninstitutionalized adults aged 40 years and older, the prevalence of peripheral neuropathy in people with diabetes ($n=419$) was 28.5% (95% CI 22.0–35.1). The prevalence of peripheral neuropathy in people with diabetes was almost twice as high as in those without diabetes (14.8% (95% CI 12.8–16.8)) [15]. Another study utilizing NHANES data found that the incidence of peripheral neuropathy was higher in people with undiagnosed (16.6%) and diagnosed (19.4%) diabetes when compared to people without diabetes or with impaired fasting glucose levels between 100 and 125 mg/dL [16]. In the mid-1990s, the annual incidence of peripheral neuropathy was nearly equivalent between genders, but more recent data have shown a growing gap with male incidence climbing [17] (Fig. 1.2).

Although many manifestations of neuropathy may go unrecognized by the patient, autonomic neuropathy is perhaps the most overlooked in the diabetic limb. In addition to contributing to impaired vasoregulation, it also may result in changes to the texture and turgor of the skin, such as dryness and fissuring. Dysregulation of local perspiration may contribute to increased moisture and increase the risk of fungal infections. With increased stiffness within the skin, areas of friction are less flexible and hyperkeratotic lesions may develop. Untreated, these lesions may progress with respect to thickness and induration, and exert increased pressure on deep tissues with resultant ulceration [18, 19].

Another form of neuropathy that influences the diabetic limb is reduced motor function. Frequently, this targets the intrinsic musculature of the foot resulting in joint instability. As innervation decreases, muscle wasting is observed. Over time, these imbalances lead to flexible deformities that become progressively more rigid. Rigid deformities are subject to greater pressure and predispose patients to ulcer formation [9].

Perhaps the most commonly recognized form of neuropathy among patients with diabetes is sensory neuropathy, resulting in the loss of sensation beginning in the most distal part of the extremity. This may manifest as an inability to detect temperature changes, vibration, proprioception, pressure, and, most seriously, pain. Some patients have a form of painful sensory neuropathy that includes symptoms, such as burning and tingling, known as paresthesias. This also contributes to the risk of ulcer formation as they may be unaware of pain associated with smaller injuries because of the persistent neuropathic pain [9]. The prevalence of painful DPN is difficult to truly measure and define. NHANES estimated that 10.9% of adults with diabetes suffered from symptomatic DPN. Symptomatic DPN was defined as painful sensations, tingling, numbness, or loss of feeling. A population-based study through the Mayo clinic found that 20% of their diabetic cohort had painful DPN [10]. In the UK, the prevalence of chronic painful DPN was found to be 16.2% [20] and the incidence, through a UK research database, was 15.3/100,000 patient-years (95% CI 14.9–15.7) [21]. Although there is a lack of high-quality data available from a population health perspective, the prevalence of DPN



PAD: Peripheral Arterial Disease.

Fig. 1.2 Rates of neuropathy and PAD by gender according to hospital discharges [17]

is believed to increase with the duration of diabetes, poor glucose control, age, and smoking [12, 22, 23]. There is significant variability in the prevalence of DPN reported in the literature. This is most likely attributable to differences among each study's population, geographic location, time period evaluated, definition of neuropathy, method of diagnosis, and source of data (i.e., patient self-report, billing codes, medical records, physician reports). It is important to note that peripheral neuropathy is likely the most important risk factor underlying the majority of diabetic lower extremity complications. Strong associations have been identified between peripheral neuropathy and DFU, diabetic foot infections, amputations, Charcot arthropathy, and surgical site infections over the last several decades [24–38] (REFS).

Peripheral Vascular Disease

Consequences of the compromised vascular system in diabetes can be among the most devastating complications. Both macro- and microvascular diseases are believed to contribute to the consequences of peripheral vascular disease, resulting in the inability of the dysvascular or ischemic limb to heal itself properly. Small injuries may progress to larger wounds because of reduced healing capacity. Delivery of systemic antibiotics can be compromised and leave infections uncontrolled. Among patients with diabetes, all blood vessels regardless of size and function are affected [39]. The prevalence of peripheral arterial disease (PAD) is higher in people with diabetes compared to the general US population. NHANES found that the prevalence of PAD was 4.5% (95% CI 3.4–5.6) in the general population but increased to 9.5% (95% CI 5.5–13.4) in persons with diabetes [15]. Figure 1.2 also illustrates that the largest disparity between genders was in 1996, and since that time the gap has reduced substantially with near equality of the rate per 1000 diabetic patients in 2003 [17]. Studies have shown that peripheral vascular disease develops at a younger age among patients with diabetes as compared to the general population [40]. In one large population-based study, over half of diabetic subjects were found to have absent pedal pulses, a common sign of impaired vascular function [40]. Another study found that in patients with nonpalpable pulses, the relative risk of ulceration was 4.72 (95% CI 3.28, 6.78) as compared to a normal exam with all four pulses palpable [41]. Ankle-brachial index (ABI), despite recognized limitations in the diabetic population, has also been used in diabetic screening. In patients with an ABI <0.90, the relative risk has been reported to be 1.25 (95% CI 1.05, 1.47) for developing an ulcer vs. diabetic patients with a normal ABI [42]. In the widely published EURODIALE DFU study from Europe, patients with PAD had a 71% increased risk for failure to heal their ulcers and a 61% increased risk for infection compared to those foot ulcer patients without PAD [43] (Prompers). The Society for Vascular Surgery published a clinical practice guideline in 2016 that reviews the association of PAD with diabetic foot complications and appropriate management strategies for the ischemic diabetic foot [44] (Hingorani).

Musculoskeletal Deformity

Musculoskeletal deformities play an important role in the diabetic ulcer pathway. The presence or absence of a deformity, such as a hammertoe or bunion, predisposes the structures to increased pressure and friction. As noted above, motor neuropathy may contribute to such deformities, but other diabetes-associated complications such as glycation of collagen have also been indicted [45–47]. In a population-

based study of a nationally representative sample, the prevalence of LED has been found to be significantly higher in those with diabetes (30.2% (95% CI 22.1–35.1)) compared to those without diabetes (18.7% (95% CI 15.9–21.4)) in the USA [15].

The prevalence of foot deformity in people with diabetes is not known, but the presence of foot deformity has been shown to increase the risk of developing a foot ulcer. One study found that 63% of patients who developed an ulcer had a fixed deformity beforehand [48]. In one large population-based study of diabetes, the relative risk of ulcer occurrence was 2.56 (95% CI 2.04, 3.22) among patients with deformities as compared to individuals with no or few deformities [41]. Boyko et al. identified the presence of an abnormally shaped foot as carrying a relative risk of 1.93 (95% CI 1.07, 3.48) for ulceration [42]. A study by Mason and associates found that patients with diabetes had similar proportions of deformities to rheumatoid arthritis patients [49]. Foot deformities, including limited joint mobility, lead to higher plantar foot pressures and these consequently often lead to an increased risk for DFU [38, 50–52]. Restriction of ankle joint dorsiflexion caused by a tight Achilles tendon (equinus deformity) has been found prevalent in neuropathic diabetic patients and is also associated with increased risks for forefoot ulcers [51, 53–56].

Metabolic and Systemic Risk Factors

In addition to specific risk factors noted above, the prevalence of LED is also increased among patients with several modifiable systemic risk factors. Cross-sectional and cohort studies have established that better glycemic control is associated with reduced risk of lower extremity amputation (LEA), but this has been difficult to demonstrate in randomized trials [57, 58]. Nonetheless, one systematic review investigating the associations between glycated hemoglobin and amputation found an overall relative risk for LEA of 1.26 (95% CI 1.16–1.36) for each percentage increase in HgbA1c [59]. The American Diabetes Association recommends that many complications, including LED, may be reduced by maintaining HbA1c <7.0%, blood pressure <130/80, HDL cholesterol >50 mg/dL, normal weight (BMI 18.5–25 kg/m [3]), and not smoking. Using data from 1999 to 2004 NHANES, Dorsey et al. reported that diabetic patients with LED were less likely to have met HbA1c (39.5% vs. 53.5%) and HDL cholesterol targets (29.7% vs. 41.1%) than patients without LED. Among non-Hispanic (NH) Blacks with LED, it was also noted that systolic and diastolic blood pressure was significantly less likely to be controlled than among non-Hispanic Whites [60]. A recent review on diabetic foot ulcers also indicates the important role played by elevated glycated hemoglobin levels on their recurrence and the importance of maintaining optimal glucose control in this regard [26].

The Perfect Storm

Thus far, the presence of individual risk factors leading to ulceration has been described, but this fails to capture the interaction of these risk factors in the clinical setting. Reiber and colleagues have proposed a widely accepted causal pathway, which incorporates the relationships between risk factors and ulcerations [48]. They advocate that the singular presence of individual risk factors represents a component but not a sufficient cause for acute ulceration. Rather, they found that the presence of two or more risk factors increased the risk of ulceration between 35 and 78% depending on the component risk factors. Furthermore, they noted that a "clinical triad" comprising neuropathy, minor foot trauma, and foot deformity was present in more than 63% of cohort patients who developed an ulcer. Peripheral neuropathy as represented by loss of protective threshold was evident in 78% of ulcer pathways, while peripheral vascular disease was a component cause in 35% of the pathways. Foot deformities were identified as a component cause in 63% of ulcer pathways [48].

Ulcerations

Schaper defined a diabetic foot ulcer as any wound below the ankle with disruption of the integument, including gangrenous tissue [61]. The annual incidence of diabetic ulceration has been reported to be between 1.9 and 4.1% in population-based studies of at least 1000 subjects [41, 62, 63]. One study noted that the prevalence of foot ulcerations was 7.7% among diabetic as compared to 2.8% among nondiabetic individuals [15]. Singh and associates reported that the lifetime risk of developing an ulcer among diabetic patients ranges between 15 and 25% [64]. Over a decade, the number of discharges in the USA related to an ulcer increased from 241,000 in 1994 to 347,000 in 2003 [17] (Fig. 1.3). Healing wounds can be difficult, and the longer the wound is open the greater the likelihood of a complication, such as infection. Even if a wound heals, the risk of recurrence is high. Apelqvist et al. reported that 70% of patients with diabetic foot ulcers will suffer reulceration within 5 years [65]. In a more recent study from this same Swedish group, 617 patients with healed DFU were followed for ulcer recurrence over the subsequent 24 months. They found that 262 patients (42%) developed a new or recurrent foot ulcer within the 2 year time period [66]. In other studies, ulcer recurrence rates have been found to range from 28% at 12 months [67] to 100% at 40 months [68].

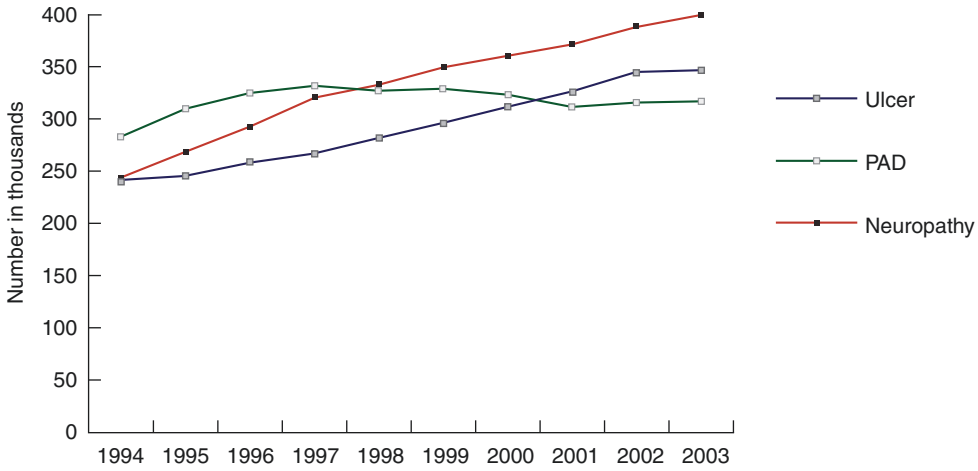


Fig. 1.3 Hospital discharges for lower extremity conditions (PAD, Neuropathy, and Ulcer with associated pathology) with a diagnosis of diabetes [17]

In a cohort of 370 patients presenting with diabetic foot ulcers, only 62.4% primarily healed all wounds. Of those patients who healed their wounds, 40.3% developed a subsequent wound after a median of 126 (14–903) days. Using Kaplan–Meier survival analysis, the authors found that the greatest period of risk for reulceration was within the first 50 days after healing. Moreover, they noted that the proportion of patients that had avoided early reulceration and remained ulcer free was 63 and 55% at 12 and 24 months, respectively [69]. Figure 1.4 shows the results of five prospective studies on primary healing, amputation, and death in patients with a diabetic foot ulcer [70–73].

Frequently, the hazardous perceptions of diabetic foot ulcers are attributed to their association with infection and amputation. Research in the past decade has indicated that the presence of an ulcer itself is associated with mortality risks. One such study found that the overall 5-year mortality rate was 44% following ulceration [72]. Even after removing patients who had gone on to amputation, the mortality rate was 43% after 5 years. Another important consideration raised by Moulik et al. was the influence of ulcer etiology on outcomes. Specifically, it was found that individuals with ischemic ulcers had a higher 5-year mortality rate and shorter median time to death than purely neuropathic and mixed neuroischemic ulcers. Similarly, the 5-year amputation rate was significantly lower in patients with a purely neuropathic ulcer than either group with an ischemic etiology [72]. Gershater et al. further explored the impact of ulcer etiology on outcome, with the results of both studies noted in Table 1.1 [71, 72].

As evidenced in Table 1.1 and Fig. 1.4, the development of a foot ulcer is a major risk factor for LEAs [74]. In fact, it has been proposed that foot ulcers precede 84% of diabetes-related amputations and are a common diabetes-related cause of hospitalization [75, 76]. Moreover, patients with neuropathic diabetic foot ulceration have a 7% risk of amputation in the next 10 years [77].

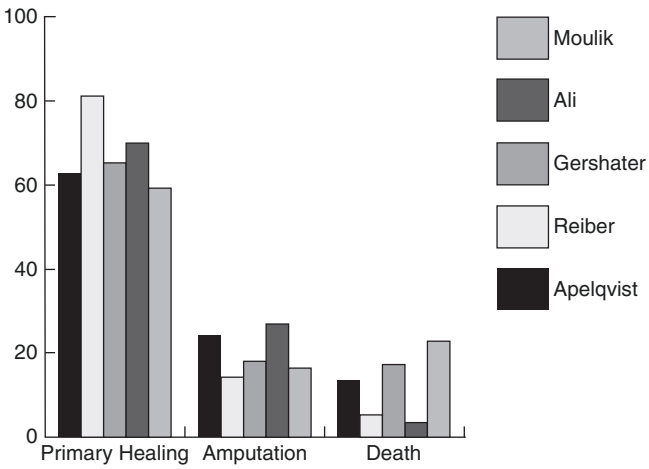


Fig. 1.4 Prospective studies on primary healing, amputation, and death in patients with a diabetic foot ulcer [70–73]

Table 1.1 Clinical outcomes of diabetic foot ulcers by etiology [71, 72]

Study type of ulcer	Primary healing (%)	Amputation (%)	Death (%)
Gershater (n = 2480)			
Neuropathic	79.4	9.5	11.1
Neuroischemic	44.4	30.1	25.5
Moulik (n = 157)			
Neuropathic	65.4	9.6	25
Neuroischemic	59	23	18
Ischemic	29	25	46

Amputations

One of the more devastating and feared outcomes of diabetic complications is lower limb amputation. By definition, it is the failure of limb preservation methods and represents the most severe consequence of diabetes on the lower extremity. Too often it can be a necessary outcome of life-

saving efforts to manage necrotizing infections in the diabetic foot or leg. While risk factors for amputation vary from study to study, nonhealing DFUs, PAD, gangrene, and infection are generally considered to be the most consistent predictors for amputation in the diabetic population [43, 78–83]. The leading cause of nontraumatic LEAs in the United States is diabetes, comprising about 60% of all such operations [15]. In 2010, approximately 73,000 nontraumatic amputations were performed in diabetic adults aged 20 years or older [5]. This is actually an underestimation since this data is sourced from public hospital databases and excludes those procedures performed in VA, military, and Indian Health facilities. Some estimates have stated that the likelihood of amputation is 10–30 times higher among patients with diabetes than in the general population of the USA [84–89]. According to the Agency for Healthcare Research and Quality (AHRQ) National Quality Health Report, in the year 2007, the age-adjusted incidence of amputations attributable to diabetes was 33.6 per 100,000 among Americans of the age 18 and older [90] (Fig. 1.5). Among Medicare beneficiaries with diabetes, the annual incidence of LEA was 0.5% in 2006 and 2007 and 0.4% in 2008. Those beneficiaries with diabetes and PAD, however, have a fourfold higher risk of LEA with an incidence of 1.8% in 2008 [91]. The US Department of Health and Human Services' Healthy People 2010 report states an objective of reducing diabetic amputations from the 1998 baseline of 6.6 per 1000 to a target of 2.9 per 1000 patients with diabetes. The Healthy People 2010 Midcourse review reported that at the time of the review 49% of the target reduction had been achieved, which translated to an incidence of 4.7 per 1000 patients [92]. Several changes in the quality of care have occurred in the past decade including the adoption of the team approach [93–95] that may have led to these improvements. These are detailed extensively in another chapter. The incidence of amputations has persistently trended down despite an increase in the prevalence in diabetes overall [92, 96]. Despite these improvements, differences persist along demographic lines, including age, race, and gender. The causes of these inequalities are beyond the scope of this chapter but are included to facilitate a more complete epidemiologic understanding by the readers.

Gender Disparities

Numerous studies have provided support that men have a higher risk of amputation than women even after controlling for factors, such as age. This difference has been observed in amputations related to trauma as well as diabetes. Among individuals with diabetes, the risk of amputation appears to be two times greater in men [97]. As of 1999, the age-adjusted incidence was 4.1 per 1000 for females and 9.2 per 1000 in males. Six years later, in 2005, the age-adjusted rates were 2.6 per 1000 and 5.6 per 1000, respectively [98]. In 2008 the annual incidence of LEA in the Medicare population was 0.6% for males and 0.3% for females with diabetes [91]. Although the overall incidence has decreased for both genders—37% reduction for women and 39% for men—the gap between the groups persists [98]. The disparity between men and women persists even along racial and ethnic lines (Fig. 1.6). Using data from 2004, White non-Hispanic males have a rate 2.4 times higher than females. In terms of gender disparity, this is followed by Hispanics at 2.1 and Asians/Pacific Islanders at 1.7, while Black/NH men have only a 1.56 higher incidence of amputations relative to Black/NH women [99].

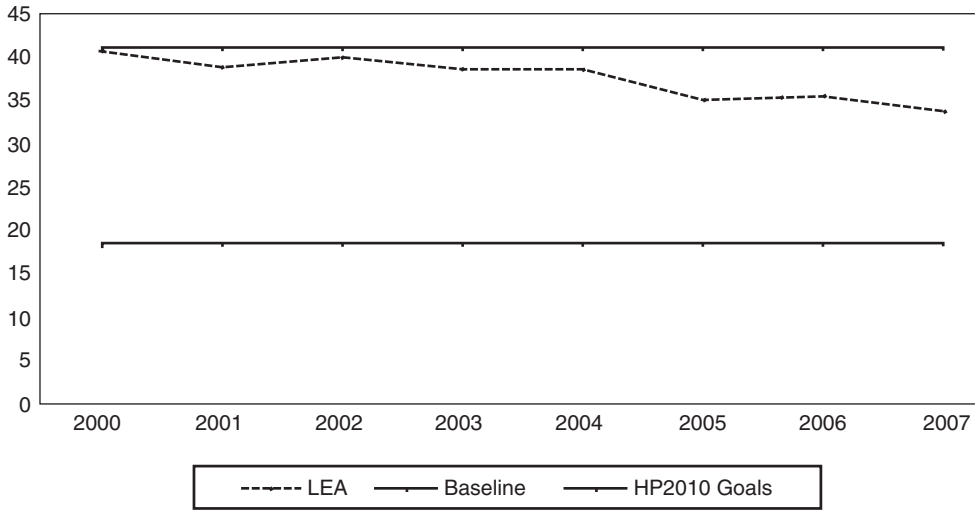


Fig. 1.5 Diabetes-associated lower extremity amputations per 100,000 population [90]

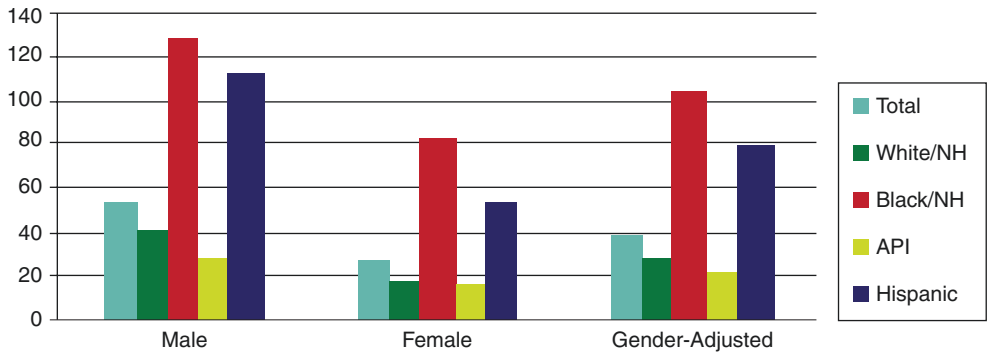


Fig. 1.6 Race-specific age-adjusted rates of amputation by gender per 100,000 general [99]

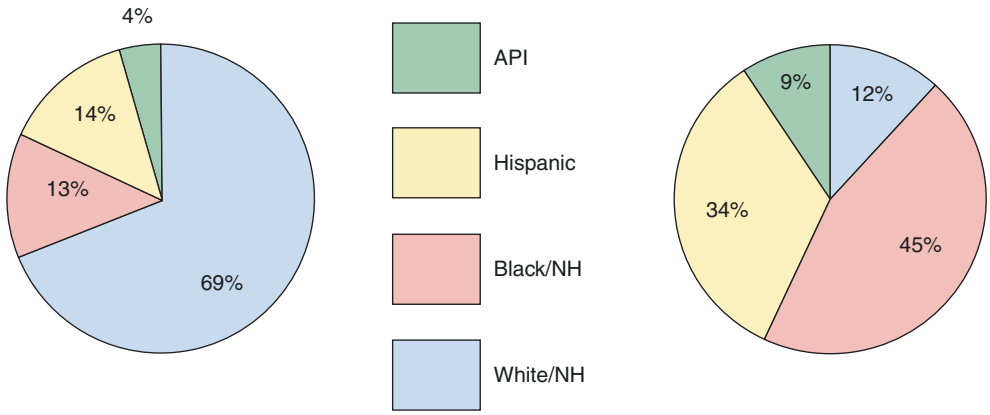


Fig. 1.7 *Left*: The US population projections by race and ethnicity [100]. *Right*: Proportion of diabetic LEA risk by race and ethnic divisions [99]

Racial and Ethnic Disparities

Differences in the incidence of diabetic amputation vary substantially among racial and ethnic groups, although the overall incidence rate has decreased over time. The racial and ethnic divisions that follow are broadly defined in the manner most frequently employed by the CDC, AHRQ, and other monitoring agencies. Because these sources are updated on an annual basis, they provide readers with a consistent reference for these figures. Incidence is discussed in terms of the rate per 1000 persons with diabetes and the rate per 100,000 total population. Although the former calculation is the more informative from an epidemiologic perspective, it is also less accurate because of estimates made about the prevalence of diabetes. In most population-level studies, white non-Hispanic individuals frequently serve as the reference group in the USA. With this common reference, the risk of a White/NH diabetic patient would be equal to 1.0.

Despite having the smallest disparity between genders, Black/NH diabetic patients have the highest incidence of LEAs in the studied population. The incidence was 5.7 per 1000 between 2004 and 2006, a rate 2.3 times higher than the 2.5 per 1000 among White/NH during the same time period. If the general population is used as the denominator, then the risk is 3.8 times greater than White/NH Americans. The incidence rate attributed to Hispanics and Latinos was twice that found in White/NH per 1000 diabetic patients, making them the second highest at-risk racial group. This ethnic group also has the largest gap between genders among minority populations. Finally, Asians and Pacific Islanders have a relative risk that is 23% lower than White/NH diabetics and also boast the second smallest disparity between males and females. As a group, Asian and Pacific Islanders had achieved 87% of the Healthy People 2010 goal by 2004. The US census estimates by racial and ethnic proportions in 2004 [100] and the proportion of risk among these categories [99] are shown in Fig. 1.7.

Socioeconomic Differences

Gender and racial/ethnic differences have been presented above, but beyond the scope of clinical characteristics are regional and socioeconomic determinants, which have also been reported as a source of disparate outcomes [101]. Socioeconomic status is a term that attempts to capture an individual's capacity to function within society. This is often measured using their level of education, annual income, or community of residence. Several studies support the proposal that lower socioeconomic status carries a higher likelihood of amputation [97, 102]. This impacts the overall health of an individual in many ways. Lower educa-

tion can reduce an individual's health literacy, the understanding of one's health, and behaviors that promote a healthy lifestyle. It may also impair early recognition of pathology before it becomes limb threatening. Annual income may impact the means to seek or obtain care or purchase supplies/medications to carry out treatments prescribed by the medical team. Lower income may also reflect an occupation that does not permit the absence from work in order to seek care.

The wealth of a community also can contribute to limitations in access to care and resources that can be directed to remove obstacles. An individual with an ulceration living in a wealthy community, where a specialized wound center was present and easily accessible to provide treatment, would be more likely to obtain care than an individual living in a resource-poor community, where the treatment options may be more limited and less effective in reducing the likelihood of progression. A frequently used proxy for community resources is the median income of a given zip code. Again, comparing data from 2004, the incidence of amputations was 33% higher in communities where the median income was less than $25,000 as compared to the incidence where the median income was $25,000–$34,999. This difference becomes even more substantial when compared to communities with a median income of $45,000 or more, where the incidence is 2.4 times greater in the under-$25,000 categories. Since the median income for the USA was $44,389 in 2004, these data suggests that the age- and gender-adjusted relative risk of amputation is between 25 and 240% higher for communities where the median income is below the national median than for communities where the median income is above the national median.

Between 2000 and 2007, the first quartile, representing the lowest median income, has realized a 23.4% reduction in incident diabetes LEAs ($p=0.0003$) (Fig. 1.8). Despite this positive outcome, as of 2007, the incidence in the highest quartile was 55% lower than that in the first quartile ($p<0.0001$) [103].

Outcomes

Amputation outcomes most often vary based on the level and location of procedure along with the corresponding postamputation complications. Generally, minor amputations are considered as limb salvage procedures and are associated with longer survival than major amputations. Each level carries with it different consequences ranging from recurrent foot ulcerations to death. The two most pressing consequences are those of subsequent amputation and death. One study found that the overall reamputation rates were 26.7, 48.3, and 60.7% after 1, 3, and 5 years following the index amputation, respectively. In general, the more proximal the amputation, the higher the likelihood of a more severe complication [104]. During the first 12 months following a toe amputation, the risk of another amputation is 22.8% on the ipsilateral side and 3.5% on the contralateral side. Over a 5-year period, the risk increases to 52.3 and 29.5%, respectively. For midfoot amputations, 18.8% of patients required another amputation on the same side during the first year, and 9.4% required an amputation on the opposing limb during that same time. After 5 years, the incidence of amputation increases to 42.9% on the same limb and 33.3% on the contralateral limb. Individuals with either a transtibial or more proximal amputation had a reamputation proportion of 4.7 and 13.3% of the same extremity after 1 and 5 years, respectively. Surprisingly, a subsequent amputation of the contralateral limb occurred in 11.6% after 1 year and 53.3% after 5 years. It would be expected that a higher occurrence of additional amputations would be seen after distal procedures given the presence of more at-risk structures. A more recent study of 116 Veterans who underwent a forefoot amputation found that 49% underwent ipsilateral reamputation within 3 years after the initial procedure, with 79% having the reamputation within the first 6 months [79]. These findings support an approach using frequent surveillance, careful monitoring, and postamputation education to reduce the risk of subsequent amputations [86, 105].

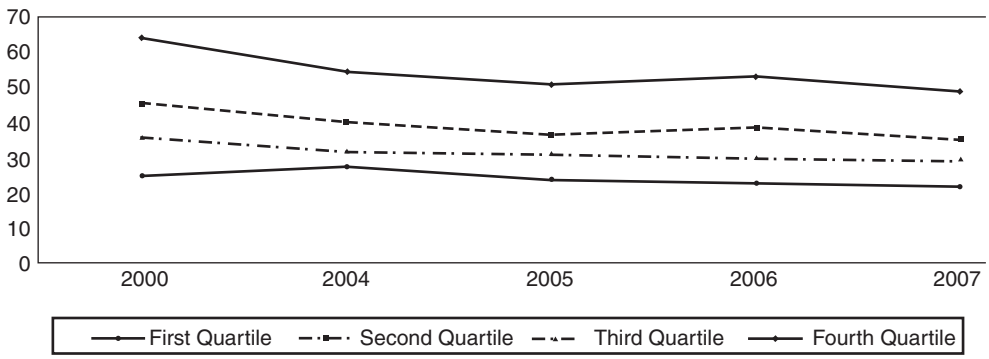


Fig. 1.8 LEA incidence by zip code median income [103]

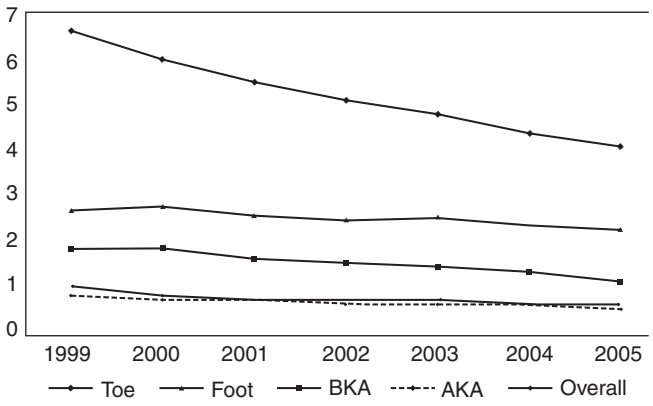


Fig. 1.9 Amputation rate per 1000 patients with diabetes [17]

An important distinction to make is the level of the amputation performed. The clinical relevance is detailed in the next section. Studies often distinguish between minor amputations (ICD-9 84.11 (toe), 84.12–84.13 (transmetatarsal), 84.14) and major amputations (84.15–84.16 (transtibial), 84.17–84.19 (transfemoral)) [86, 106]. Although this is not universally the protocol, it is frequently encountered. Figure 1.9 demonstrates that toe amputations are the most frequent, followed by below-the-knee amputations (BKAs). The trends show that a decline in the incidence is evident at all levels of amputation per 1000 patients with diabetes [104, 106].

Mortality

A direct causal relationship between amputation and short-term mortality has not been proven, but a strong association between these variables has been shown in several studies [72, 107, 108]. One proposed mechanism is that the postamputation exertion of gait stresses the cardiovascular system and increases the risk of a fatal cardiac event.

Amputation is not a benign outcome for either diabetic or nondiabetic patients. One study noted that the 1-, 5-, and 10-year mortality rates for nondiabetic individuals were 27.3%, 57.2%, and 77.1%, respectively. The study also noted that diabetic mortality was reported to be 32.8% after 1 year, 68.1% after 5 years, and 91.6% after 10 years. In the observed populations, the gap between the respective groups increased mortality rate from 5.5 to 14.5%. The authors concluded that diabetic patients had a 55% greater risk of death following amputation than nondiabetics, and that median survival was 27.2 months and 46.7 months, respectively [86]. More recently, a very large study of diabetic patients in the UK was reported in 2015 that specifically investigated the association between LEA and risk for death. In this study of more than 416,000 persons with diabetes, 6566 (1.6%) had an LEA and 77,215 persons died during the 10-year study period. After adjusting for all known covariates that might also predict death in this population, there was a greater than twofold independent risk for death in patients who had undergone LEA (HR 2.37 (95% CI 2.27–2.48) [109]. Hence it seems that diabetes-related LEA portends a significant risk of death even after controlling for major cardiovascular risk factors when compared to those diabetic patients not suffering lower extremity amputations.

As noted with the risk of reamputation, the risk of mortality is also influenced by the level of the index amputation. Within 1 year of the index amputation, mortality rates for diabetic patients were 6.6% after digital amputations, 4.4% after ray amputations, 10.5% after midfoot amputations, and 18.2% after a major amputation. Extending this to 5 years from the initial amputation, toe and ray amputations had mortality rates of 26.2% and 15.8%, respectively. Five-year mortality after a major amputation was found to be 36%, while midfoot amputations carried a risk of 21% [104].

Perioperative Mortality

Perioperative mortality has been reported to be quite high following amputation. Mortality rates have ranged between 5.8 and 23% during the first 30 days following amputation [97, 110–114]. Patients requiring a guillotine amputation secondary to sepsis have a particularly high perioperative mortality rate of 14.3% [48]. The most frequently cited 30-day mortality causes have been cardiac events and sepsis [97]. Short-term mortality following amputation is primarily related to cardiac events, with rates ranging from 28.5 to 52.2% [104, 110]. Sepsis is the second most frequent cause of death, with rates ranging from 14.2 to 26.1% [104, 110, 115]. The level of amputation again has an influence on this outcome. Two distinct studies demonstrate similar 30-day mortality rates following above-the-knee (AKA) or below-the-knee amputations. Subramaniam et al. reported 17.5 and 4.2% mortality, while Stone et al. reported 17.6% and 3.6%, respectively [114]. The results by Stone et al. were more comprehensive and demonstrated a trend of increasing perioperative mortality as amputations became more proximal starting at the metatarsals and ending at the hip [116].

Cost of Lower Extremity Disease in Diabetes

Cost to the Health Care System

Thus far, this chapter has covered the epidemiologic aspects of the at-risk foot. The remaining portion focuses on the costs attributable to these conditions. Boulton et al. commented on the substantial economic burden that the diabetic foot places on the afflicted patient and the health care system, although they recognized that most estimates fail to account

for preventive care, lost productivity, and rehabilitation. They further proposed that if these aspects were also added to the current estimates as much as 20% of diabetes costs could be associated with diabetic foot ulcers [117]. The excess costs are primarily attributable to more frequent hospitalization, use of antibiotics, and need for amputations and other surgical procedures [118].

Harrington and colleagues examined excess costs attributable to patients with diabetic foot ulcers vs. those with diabetes alone. Among the Medicare population sampled, they found that the direct costs per patient per year were $15,300 among patients with ulcers vs. $5200 for patients without an ulcer [119].

Similar findings were noted in a health maintenance organization (HMO) population, where diabetic patients without ulcers had a cost per patient per year of $5080 while it remained substantially higher for patients with an ulcer at $26,490 per patient per year [63, 120]. Costs also vary considerably based on ulcer grade. In a large insurance claims database, Stockl et al. observed that the cost of an ulcer episode ranged from $1892 for a level 1 ulcer to $27,721 for level 4/5 ulcers [121]. Overall, inpatient hospital charges comprised 77% of total costs.

Costs can also be examined in the context of clinical outcomes, and significant differences exist among patients who achieve primary healing vs. amputation. Apelqvist et al. [107, 122, 123] found that the cost of primary healing was $6800 per admission while Holzer et al. [124] found a smaller cost of $1920 per episode; however, the cost jumped substantially if complicated by osteomyelitis ($3580). In the same study, patients requiring an amputation had an associated cost of $15,790 per admission. Further cost comparisons can be made between patients who required amputation and those who did not need an amputation. The Apelqvist study reported that the average cost of amputation per admission was $45,870. Differences in cost via amputation level are also present, where the major amputations have been 1.5–2.3 times higher than minor amputations [107, 122, 123, 125]. Many of these study costs were drawn from different time periods, so for ease of interpretation Table 1.2 demonstrates currency values to 1998 and 2010 equivalents [126].

According to data from the national inpatient sample population, more proximal amputations have been associated with higher costs and longer lengths of stay (Table 1.3). This is likely attributable to the increased morbidity and mortality associated with major amputations. In 2008, the average length of stay was 47% longer after a major amputation as compared to the mean stay after a toe amputation. Similarly, the mean charges were 53% higher after a major amputation relative to average toe amputations [127]. A comparison of length of stay and charges associated with ulcerations and amputations by insurance payer can be seen in Table 1.4 [103].

Table 1.2 Costs of various diabetic foot complications adjusted to the US currency in 1998 and 2010

	1998 ($)	2010 ($)
Diabetes without ulcer	5402.17 [2] 5433.33 [3]	7225.35 [2] 7267.03 [3]
Diabetes with ulcer	15,894.84 [2] 28,332.48 [3]	21,259.20 [2] 37,894.43 [3]
DM ulcer with primary healing	8659 [4, 9]	11,581.33 [4, 9]
DM ulcer with amputation	43,270.44 [4, 9] 2452 [10]	57,873.82 [4, 9] 3279.53 [10]
DM major amputation	66,215 [4, 9] 45,343 [11]	88,561.95 [4, 9] 60,645.85 [11]
DM minor amputation	43,800 [4, 9] 19,996 [11]	58,582.10 [4, 9] 26,744.47 [11]

Table 1.3 Charges to hospitals for patients with diabetes by amputation level, 2008 [103]

		Diabetes with complications		Overall	
ICD-9	Amputation	Length of stay	Average charge ($)	Length of stay	Average charge ($)
84.11	Toe amputation	8.3	45,509	8.4	45,468
84.12	Amputation through foot	11.8	69,064	12.3	73,160
84.15	Below-the-knee amputation	12.2	68,542	12.8	77,577
84.17	Above-the-knee amputation	12.6	69,380	13.1	79,982

Cost-Effectiveness of Prevention

Most physicians and patients agree that prevention of lower extremity ulceration, infection, and amputation is the most desirable clinical strategy, and several studies have shown that this approach is either highly cost-effective or cost saving. In the UK, a 2-year prospective cohort study of 2000 patients comparing a diabetic foot protection and screening program with conventional diabetes care demonstrated that only 24 patients in the protection program developed ulcers vs. 35 patients receiving conventional care. More importantly, only 7 of the patients with ulcers in the specialized program progressed to amputation, whereas 23 progressed in the conventional care group ($p<0.01$). The total cost of the screening program was only £100 per patient per year while producing a savings of 11 amputations in 1000 patients at a cost of £12,084/amputation [128]. A retrospective cohort study from Austria using a Markov model to estimate long-term costs and outcomes in a dedicated screening program compared with conventional care similarly concluded that the screening program would reduce costs by 29.8% for mild (grade A) ulcers and by 49.7% for severe (grade D) ulcers, primarily due to lower amputation rates [129]. In a systematic review from the CDC on the cost-effectiveness of inter-

Table 1.4 Ulcer and amputation charges by hospitals for patients with diabetes, 2005 [103, 127]

		Medicare		Medicaid		Private	
		Length of stay	Average charge ($)	Length of stay	Average charge ($)	Length of stay	Average charge ($)
DRG	*Condition*						
271	Skin ulcers in diabetes with complications	9.8	26,937[a]	6.4	19,787	6.8	19,885
	Skin ulcers in diabetes without complications	9.8	25,803	7.8	25,429	8.2	25,395
199	Chronic ulcer in diabetes with complications	12.4	39,343	10.0	35,126	9.4	33,317
	Chronic ulcer in diabetes without complications	11.4	32,999	10.1	30,530	8.2	27,886
157	Lower extremity amputation in diabetes with complications	10.8	47,110	11.7	47,493	9.4	42,586

[a]Charges do not include professional fee

ventions to prevent diabetes and its complications, the use of comprehensive foot care to prevent ulcers was one of the few interventions found to be cost saving [130].

Evaluation of changes in quality of life, as reflected in cost–utility analysis, has shown similar results. Ortegon et al. used a Markov model to estimate lifetime risk of developing foot disease among newly diagnosed patients with type 2 diabetes receiving optimal foot care guidelines, intensive glycemic control, or standard care [131]. In all simulations using a wide range of assumptions in the sensitivity analysis, use of guidelines for foot care resulted in longer life expectancy, improved quality of life, lower incidence of foot ulcers, and fewer LEAs when compared with standard care. Most simulations demonstrated that the costs were less than $25,000 per QALY gained compared to standard care. The best results were obtained when foot care guidelines were combined with intensive glycemic control, with a cost of $7860 per QALY gained [131].

Summary

The diabetic limb is vulnerable to a variety of risk factors which have the potential to culminate in the onset of ulceration. Among patients with diabetes, the lifetime incidence of developing an ulcer is 15–25%. Wound healing may be a protracted process, and recurrent wounds are common during the first 2 months after closure [69].

Amputation is a devastating consequence of diabetic complications. Because of the intrinsic morbidity and mortality associated with amputations, diverse organizations have worked toward implementing plans to reduce amputation rates. In the USA, one such program includes the Healthy People 2010 objective to reduce the annual incidence of diabetic LEAs by 55%. By 2005, participating researchers had projected that a 29% reduction had been achieved despite an increase in diabetes prevalence by 35% during that same period [92]. The incidence for amputations consistently appears to be approximately twice as high for males as females [99]. Along racial and ethnic divisions, gender- and age-adjusted figures identify black non-Hispanics as the highest risk group and Asian/Pacific Islanders as the lowest [99]. Another high-risk group includes diabetic patients living in poor areas, where the median income is less than $25,000 annually. Although a large gap between the wealthiest and poorest quartiles persists, the largest magnitude of reduction has occurred in the poorest group [99].

The consequences of LEA can be severe, particularly in diabetic patients, where their 10-year mortality rate is nearly 20% higher than that in similar nondiabetic populations [86]. Even perioperative mortality is high, with rates between 5 and 23% reported in the first 30 days [97, 110–114]. This proportion can change depending on the level of the amputation performed. Digital and other "minor" amputations have a substantially lower mortality rate associated as compared to major amputations which may have a 5-year mortality rate of 36–69%. Subsequent amputations are also problematic, and as many as 68% of amputees will require further amputation within 5 years. This may be influenced by the level of the initial amputation, where digital amputations have a greater risk of reamputation than major amputations [105].

Health care costs associated with diabetic ulcers and amputations contribute significantly to the financial burden of diabetes. According to the US national inpatient sample, as of 2008, the total number of discharges attributed to diabetes-related amputations was projected to be 45,000. The average length of stay was 10.1 days with an inhospital mortality proportion of 1.29%. The most frequent discharge statuses were to a rehabilitation facility (37.9%), routine discharge (31.5%), or home health care (26.9%). The mean charges were $56,216 while the aggregate charges for the year 2008 had a total of $2,548,319,965. However, it is worth noting that charges and actual cost frequently are separated by a wide margin. Length of stay in the hospital was 47% longer after a major amputation than a toe amputation.

During that same time, charges following a major amputation were 53% higher than those after a digital amputation [103]. Importantly, measures aimed at preventing LED, including simple interventions such as following recommended guidelines, have been shown to be highly cost-effective in preventing ulcers and subsequent amputations.

Zimmet may have been correct to call diabetes a worldwide epidemic, as prevalence has climbed higher over the years [2, 4]. Even though the "at-risk" population has increased, the rates of limb-threatening complications have trended downward. The progressive deployment of the "team approach" to limb preservation (as Joslin had first employed) has been touted as a contributing factor, but patient education and vigilance should not be discounted for this success.

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Clinical Examination and Risk Classification of the Diabetic Foot

2

Lawrence A. Lavery, Suzanne van Asten, and Javier La Fontaine

Abstract

A consistent, thoughtful assessment of the diabetic foot is pivotal to identify patients at risk for ulceration. In this chapter, we discuss the key risk factors to screen patients for foot complications: a history of lower extremity disease, the presence of peripheral neuropathy, and foot deformities. We discuss the practical approach and background of these key risk factors and subsequently the two most commonly used classification systems for diabetic foot ulcers. Many of the risk factors for ulceration may be identified using simple, inexpensive techniques in a primary care setting. Appropriate classification of the wound becomes paramount in our efforts to document and communicate the level of risk and facilitate amputation prevention.

Foot ulceration is one of the most common precursors to lower extremity amputations among persons with diabetes [1–3]. Ulcerations are pivotal events in limb loss for two important reasons. First, they allow an avenue for infection [4], and second they can cause progressive tissue necrosis and poor wound healing in the presence of critical ischemia. Infections involving the foot rarely develop in the absence of a wound in adults with diabetes, and ulcers are the most common type of wound in this population [4]. Foot ulcers therefore play a central role in the causal pathway to lower extremity amputation [5].

The etiology of ulcerations in persons with diabetes is commonly associated with the presence of peripheral neuropathy and repetitive trauma due to normal walking activities to areas of the foot exposed to moderate or high pressure and shear forces [6]. Foot deformities, limited joint mobility, partial foot amputations and other structural deformities often predispose patients with diabetes with peripheral neuropathy to abnormal weight bearing, areas of concentrated pressure and abnormal shear forces that significantly increase their risk of ulceration [7–9]. Brand theorized that when these types of forces were applied to a discrete area over an extended period of time they would cause a local inflammatory response, focal tissue ischemia, tissue destruction, and ulceration [10]. Clearly, identification of persons at risk for ulceration is of central importance in any plan for amputation prevention and diabetes care.

Diabetic Foot Risk Classification

Preventing foot complications begins with identifying patients at risk for developing a foot ulcer. Diabetic foot screening programs are inexpensive and can be performed by technicians or nurses with very little training. In patients with signs or symptoms of loss of protective sensation caused by peripheral neuropathy, examinations should include obtaining a detailed history of ulceration and amputation of the lower extremities, and screening for the presence of peripheral artery disease and foot deformities. On top of that other patient-related factors like inadequate footwear, foot hygiene, and pre-ulcerative signs on the foot should be identified. In the updated consensus document of the International Working Group on the Diabetic Foot (IWGDF), a screening interval is added to the widely used classification system of the key risk factors [11].

Lavery et al. reported that a patient with neuropathy but no deformity or history of ulcer or amputation has a 1.7 times greater risk for ulceration compared with a patient without neuropathy [12]. Neuropathy with concomitant deformity or limited joint mobility yields a 12.1 times greater risk. Lastly, a patient with a history of previous ulceration or amputation has a 36.4 times greater risk for presenting with

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Table 2.1 The IWGDF Risk Classification System 2015 and preventative screening frequency [11]

Category	Characteristics	Frequency
0	No peripheral neuropathy	Once a year
1	Peripheral neuropathy	Once every 6 months
2	Peripheral neuropathy with peripheral artery disease and/or a foot deformity	Once every 3–6 months
3	Peripheral neuropathy and a history of foot ulcer or lower extremity amputation	Once every 3–6 months

another ulcer. These risk factors compare to the categories in the classification system promoted by the International Working Group on the Diabetic Foot [11–14] (Table 2.1) and similar classification systems described by Rith-Najarian [15] and Armstrong [16]. A comparison was made between this system and four other classification tools in a systematic review in 2011 [17]. Core values of the stratification systems were very similar, but the risk groups and number of variables that were included varied.

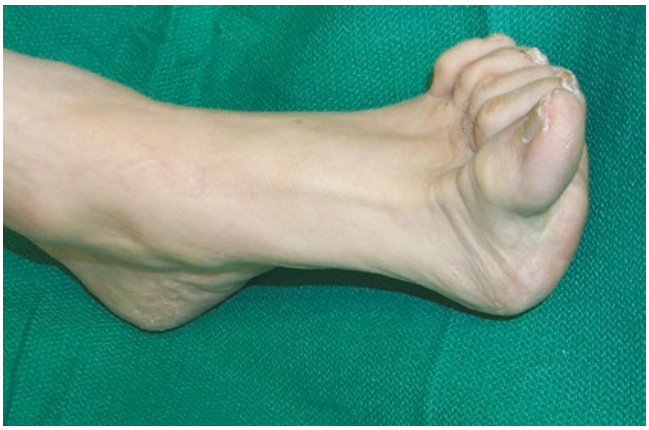

Fig. 2.1 Intrinsic muscular atrophy and foot deformity. Diabetic peripheral neuropathy also affects motor nerves, often causing atrophy of intrinsic musculature of the hand and foot. When this occurs, the extrinsic musculature work unopposed, thus causing hammering of the toes and retrograde buckling of the metatarsal heads. Thus, both the toes (dorsal) and the metatarsal heads (plantar) are more prominent and therefore more prone to neuropathic ulceration

History of Foot Pathology

History of foot disease is the strongest predictor of ulceration and amputation and the least expensive screening measure [18, 19]. It is the easiest risk group to identify, and the group most in need of frequent foot assessment, intensive education, therapeutic shoes, padded stockings, and rigorous blood glucose control. A current ulcer, past history of previous ulceration or amputation heightens the risk for further ulceration, infection, and subsequent amputation [5, 11, 17, 18]. Patients in this risk group (Risk Category 3) are about 50 times more likely to have an ulcer in the next year and 36 times more likely to have an amputation compared to patients with no neuropathy or PAD [20]. The presence of pre-ulcerative lesions such us abundant callus, hemorrhage or a blister, is a strong determinant of ulcer recurrence, especially in patients with recurrence caused by unrecognized repetitive trauma [21].

There are several potential explanations for the increased risk. Diabetic patients with a history of ulceration or amputation have all the risk factors to re-ulcerate [22, 23]. Ulceration and amputation damage the integument and the biomechanics of the foot. After healing by secondary intention, the skin and soft tissue is scarred and it may be less resilient and less pliable, so it is more prone to injury. In addition, persons with a partial foot amputation often develop local foot deformities secondary to biomechanical imbalances that may cause further foci of pressure and shear [24–26]. Structural deformities increase pressures on the sole of the foot and are associated with ulceration (Fig. 2.1). A classic example is clawing of the lesser toes and subluxation and dislocation of the metatarsophalangeal joints [26].

Peripheral Neuropathy

Neuropathy is a major component of nearly all diabetic ulcerations [27]. Loss of protective sensation is a term that is often used to describe a level of sensory loss that allows patients to jury themselves without recognizing the injury. These patients are vulnerable to physical and thermal trauma that increases the risk of foot ulceration twofold [20]. Patients with neuropathy often wear a hole in their foot much as a sensate patient might wear a hole in their stocking or shoe.

Screening for neuropathy is noninvasive, fast, and inexpensive. Several consensus documents recommend that all patients with diabetes should be screened annually for sensory neuropathy [27, 28]. There are several techniques to screen for neuropathy. The absence of protective sensation may be determined using a tuning fork, a Semmes-Weinstein 10 gram monofilament nylon wire, a calibrated vibration perception threshold (VPT) meter, or by a comprehensive physical examination.

Inspection of the feet may provide valuable clues as to the presence and severity of sensory neuropathy. Atrophy of the intrinsic muscles of the hands and feet is often a late-stage condition that is very frequently associated with polyneuropathy. When this occurs, the extrinsic muscles of the foot are unopposed, thus causing hammering of the toes and retrograde

buckling of the metatarsal heads. Thus, both the toes (dorsal) and the metatarsal heads (plantar) are more prominent and therefore more prone to neuropathic ulceration (Fig. 2.1). This condition often leads to prominent digits and metatarsal heads, and (in the face of sensory loss) has been associated with increased risk for neuropathic ulceration. Similarly, bleeding into callus is a condition which is associated with neuropathy. Patients with autonomic neuropathy may present with dry skin that is poorly hydrated.

Tuning Fork

The conventional 128 Hz tuning fork is an easy and inexpensive tool to assess vibratory sensation. The test is considered positive when the patient loses vibratory sensation while the examiner still perceives it [29]. The tuning fork is struck until it clangs, and the tip of the tuning fork is held against a bony prominence, such as the distal tip of the great toe. The patient is asked if they can feel the vibration. If they feel pressure but no vibration, they have loss of vibration sensation. In addition, the patient should be able to feel the vibration for about 20 s. If they cannot feel the vibration for 20 s, they have abnormal vibration sensation. In addition to a standard 128 Hz tuning fork, a graduated tuning (Rydel-Seiffer) fork has provided comparable results to the vibration perception testing (r, −0.90; $P < 0.001$) [30, 31]. Using the graduated tuning fork, patients indicate first loss of vibration at the plantar hallux as the intersection of 2 virtual triangles moves on a scale exponentially from 0 to 8 in a mean (AD) of 39.8 (1) seconds [32].

Semmes Weinstein Monofilament

The Semmes Weinstein monofilament is one of the most frequently utilized screening tools for identifying loss of protective sensation in the United States [28, 33]. The inability to perceive the 10 g Semmes Weinstein monofilament has been associated with large-fiber neuropathy [34, 35]. In three prospective studies, the 5.07 or 10 g Semmes Weinstein monofilament identified persons at increased risk of foot ulceration with a sensitivity of 65–91%, a specificity of 36–86%, a positive predictive value of 18–39%, and a negative predictive value of 90–95%. (Table 2.2) [18, 35, 36] The Semmes Weinstein monofilament consists of a plastic handle supporting a nylon filament. It is portable, inexpensive, easy to use, and provides excellent negative predictive ability for the risk of ulceration and amputation [37].

There are a number of important concerns regarding the Semmes Weinstein monofilament. There is wide variability in the accuracy and durability of monofilaments sold in the United States. Certain brands of monofilaments are more accurate than others [38]. Instruments made in the United Kingdom seem to have better initial accuracy and calibration [37]. Semmes Weinstein monofilaments experience material failure of the nylon monofilament and become less accurate with repeated measurements. Therefore it is important to purchase calibrated instruments and to replace them on a regular basis. In a clinical setting, it is best for the evaluator to have more than one monofilament available, as after numerous uses without a chance to "recover," the monofilament may buckle at a reduced amount of pressure, thus making it oversensitive and therefore less accurate [38]. Longevity and recovery testing results from an independent study suggest that each monofilament, regardless of the brand, will survive usage on approximately ten patients before needing a recovery time of 24 h before further use [32, 38]. Furthermore, differences in materials used in the manufacturing process and environmental factors may also change the characteristics of the monofilament [38, 39].

Testing with the Semmes Weinstein monofilament is best performed with the patient sitting supine in the examination chair with both feet level. The monofilament is applied perpendicular to the skin until it bends or buckles from the pressure. It should be left in place for approximately one second and then released [27]. The monofilament should be demonstrated on the patient's hand, so they can understand the level of pressure provided during testing. The patient should close their eyes for the foot examination. They should be instructed to say "yes" each time that they feel the monofilament and then to identify the site where they felt the monofilament. The number of sites that should be tested with monofilaments is unclear. However, because testing is noninvasive and inexpensive, the number of sites should not be a limiting factor in testing protocols. Some authorities recommend that measurements be taken at each of ten sites on the foot [40]. These include the first,

Table 2.2 10 g monofilament to diagnose sensory neuropathy

Author, year, Journal	Prevalence Ulcers %	Sensitivity / Specificity	Positive Predictive Value	Negative Predictive Value
Boyko, 1999, Diabetes Care [18]	11%	68 / 62	18	94
Rith-Najarian, 1992, Diabetes Care [35]	11%	65 / 86	39	95
Pham, 2000, Diabetes Care [36]	29%	91 / 36	34	90

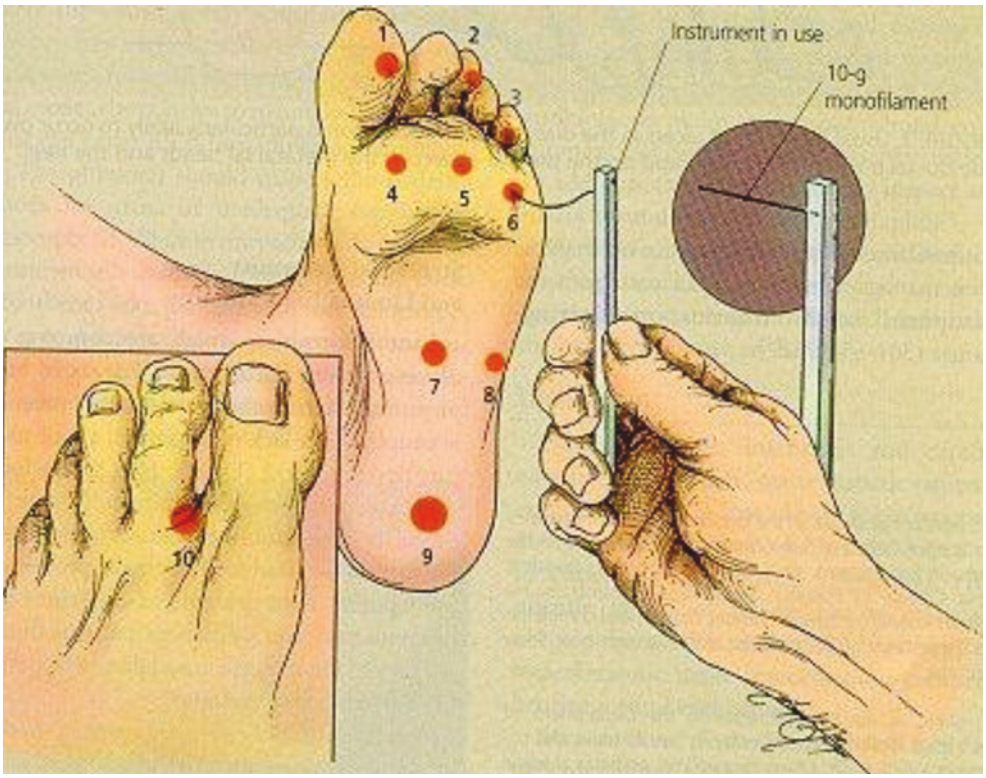

Fig. 2.2 Use of the 10-g monofilament

third, and fifth digits, plantarly, the first, third, and fifth metatarsal heads plantarly, the plantar midfoot medially and laterally, the plantar heel, and the distal first interspace, dorsally (Fig. 2.2). However, testing just four plantar sites on the forefoot (the great toe, and base of the first, third, and fifth metatarsals) identifies 90% of patients with loss of protective sensation [41].

Vibration Perception Threshold (VPT) Testing

A VPT meter is a semiquantitative tool to assess large fiber neuropathy. The VPT meter (also known as Biothesiometer or Neurothesiometer) is a handheld device with a rubber tactor that vibrates at 100 Hz. The handheld unit is connected by an electrical cord to a base unit. This unit contains a linear scale which displays the applied voltage, ranging from 0 to 100 V (converted from microns [36, 42] (Fig. 2.3). The device is held with the tactor balanced vertically on the pulp of the toe. The voltage amplitude is then increased on the base unit until the patient can perceive a vibration. A mean of three readings (measured in Volts) is generally used to determine the vibration perception threshold for each foot. "Loss of protective sensation" with VPT has commonly been considered to be about 25 V. The level of Vibration Perception Threshold testing can help to predict ulceration [43]. In a prospective cohort study Abbott and colleagues evaluated 1035 patients with diabetes, no history of a foot ulcer and a VPT greater than 25. During the follow-up period the yearly ulcer incidence was 7.2%. For every one volt increase in VPT, there was a 5.6% increase in the risk of foot ulceration [44]. VPT testing has been shown to have very good sensitivity and specificity (Table 2.3).

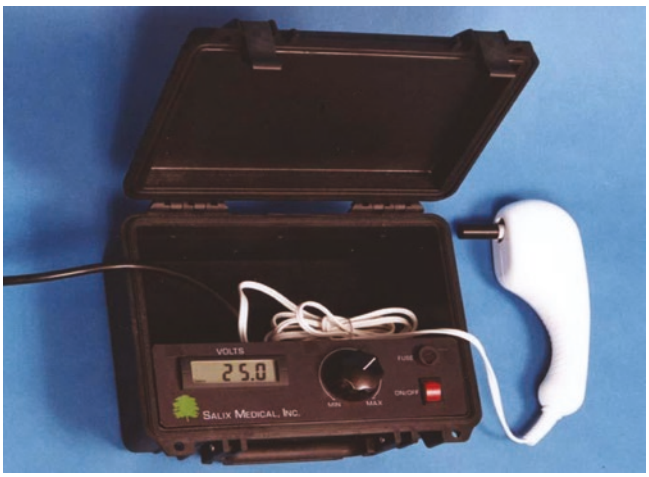

Fig. 2.3 Vibration perception threshold meter. The vibrating tactor is placed at the distal pulp of the great toe. The amplitude (measured in Volts) is increased on the base unit until the patient feels a vibration. This is termed vibration perception threshold (VPT). A VPT greater than 25 V may be an optimal combination of sensitivity and specificity for identifying clinically significant loss of protective sensation using this device

Table 2.3 Vibration Perception Threshold Testing

Author, year, Journal	Prevalence Ulcers %	Sensitivity / Specificity	Positive Predictive Value	Negative Predictive Value
Sosenko, 1990, Diabetes Care [45]	29%	83% / 87%	49%	NS
Vileikyte, 1997, Diabetes Care [46]	28%	86% / 79%	NS	NS
Armstrong, 1998, Arch Int Med [24]	33%	80% / 85%	NS	NS

NS not stated

New Neuropathy Screening Tests

Two recently described tests have been validated against the other commonly used screening test. The Ipswich Touch Test (IpTT), which assesses the ability of the patient to perceive the touch of a finger [48] and the Vibratip, a disposable vibrating stylus that can assess vibration sensation [49]. The IpTT involves lightly touching/resting the tip of the index finger for 1–2 s on the tips of the first, third, and fifth toes and the dorsum of the hallux. Direct comparison of the IpTT and monofilament testing showed almost perfect agreement, with positive predictive values indicating at-risk feet of IpTT 89%, MF 91% and negative predictive values of IpTT 77% and MF 81%. The IpTT has also been evaluated to detect reduced foot sensation in the setting of the patient's home [50]. Having a simple method to detect loss of sensation at home might improve awareness of foot disease in patients with diabetes and empower them to seek appropriate care. When activated, the VibraTip (McCallan Medical Limited, Nottinghamshire, UK) provides a stimulus of 128 Hz, mimicking the conventional tuning fork. The patient's hallux is touched twice with the rounded tip of the VibraTip, each time for approximately 1 s, while randomly activating the VibraTip on either the first or second touch. Both the Vibratip and the IpTT showed high concordance with the vibration perception threshold test ≥25 V in 83 at-risk individuals [49].

Modified Neuropathy Disability Score

Clinical assessment can be used to score the severity of peripheral neuropathy in order to identify high-risk patients. The Modified Neuropathy Disability Score is a clinical assessment scoring scheme that uses standard clinical tools. These include deep tendon reflexes of Achilles tendons, vibration sensation with 128 Hz tuning fork, pinprick, and hot and cold rods. Use of these instruments, combined into a disability score, has proven to be predictive of future diabetic foot complications [19]. In a population-based prospective study, Abbot evaluated 9710 patients with diabetes from six health districts in the United Kingdom. During the 2-year follow-up period there were 291 ulcers. Only 1.1% of patients with a Neuropathy Disability Score less than six developed a foot ulcer, and 6.3% of patients with NDS greater than six developed an ulcer [19].

Limited Joint Mobility

Neuropathy and foot deformity, when combined with repetitive or constant stress, can lead to ulceration. Characteristically, the highest plantar pressure is associated with the site of ulceration [6, 7, 51, 52]. In one study of patients with peripheral neuropathy, 28% with high plantar pressure developed a foot ulcer during a 2.5-year follow-up compared with none with normal pressure [53].

Clinicians should examine the feet for structural abnormalities including hammer or claw toes, flat feet, bunions and calluses, and reduced joint mobility to help identify pressure points that are susceptible to future ulceration. Structural deformity is frequently accompanied by limited joint mobility. Nonenzymatic glycosylation of periarticular soft tissues or tendons may contribute to limited joint motion in the person with diabetes. Neuropathy can lead to atrophy of the intrinsic muscles of the hands and feet which can cause instability at the metatarsophalangeal joint and digits [54]. Limitation of motion reduces the foot's ability to accommodate for ground reactive force and, therefore, increases plantar pressures [55–57]. Limitation of motion of the first metatarsophalangeal joint has been defined as less than 50° of passive dorsiflexion of the hallux (Fig. 2.4).

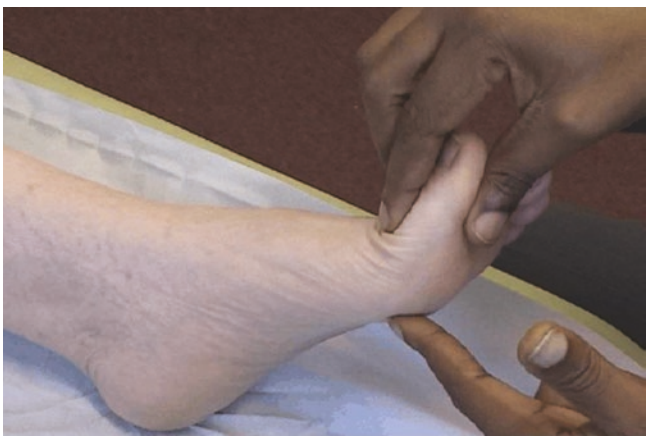

Fig. 2.4 Evaluation of first metatarsophalangeal joint dorsiflexion (limited joint mobility). Limited joint mobility is frequently encountered in patients with long-standing diabetes. This is most significant in the ankle joint (equinus) and in the forefoot. Less than 50° of dorsiflexion at the first metatarsal phalangeal joint indicates clinically significant limited joint mobility

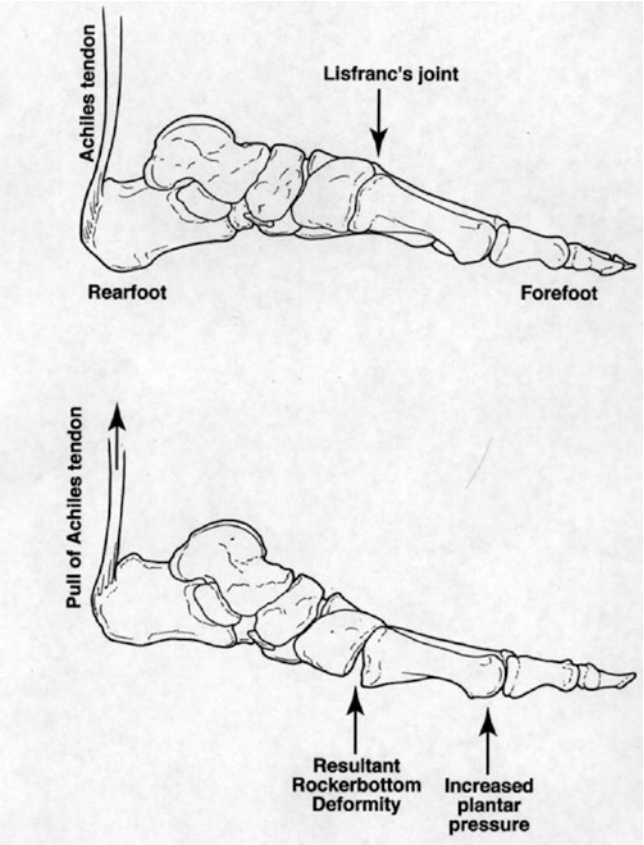

Fig. 2.5 Equinus and its relationship to elevated forefoot plantar pressure. Shortening or loss of natural extensibility of the Achilles tendon may lead to pulling of the foot into plantar flexion. This leads to increased forefoot pressure (increasing risk for plantar ulceration) and, in some patients, may be a component of midfoot collapse and Charcot arthropathy

Additionally, glycosylation may deleteriously affect the resiliency of the Achilles tendon, thereby pulling the foot into equinus and further increasing the risk for both ulceration and Charcot Arthropathy (Fig. 2.5) [58]. In a recent case control study, plantar and dorsal flexion of the feet of 87 patients with diabetes was measured and the incidence of foot ulcers was reported over a follow-up period of 8 years. Diabetes specifically reduced the plantar flexion in the feet and patients with a history of foot ulceration had significantly lower ankle joint mobility [59].

Diabetic Foot Ulcer Classification

Foot ulcers in patients with diabetes are one of the most common precursors to lower extremity amputation. Appropriate care of the diabetic foot ulceration requires a clear, descriptive classification system that can be used to direct therapy, communicate risk, and possibly predict outcome. Speaking a "common language" when communicating risk in the diabetic foot is therefore essential. A classification system, if it is to be clinically useful, should be easy to use, reproducible, and effective to accurately communicate the status of wounds in persons with diabetes mellitus. There are a variety of variables that could be included in such a system, such as faulty wound healing, compliance issues, quality of wound granulation tissue, host immunity, nutritional status, and comorbidities. However, most of these variables are difficult to measure or categorize and can complicate a system. In contrast, three relatively quantifiable factors associated with poor wound healing and amputation include depth of the wound [60, 61], presence of infection, and presence of ischemia [62].

Seven Essential Questions to Ask when Assessing a Diabetic Foot Wound

A classification system has little value if the clinician employing it does not approach each wound in a stepwise consistent, logical fashion. When employing this approach, the first four questions are useful in terms of their descriptive value. The last three questions are most useful for their predictive qualities.

1. Where Is the Ulcer Located?

Location of a wound and its etiology go hand in hand. Generally, wounds on the medial aspect of the foot are caused by constant low-pressure (e.g., tight shoes) whereas wounds on the plantar aspect of the foot are caused by repetitive moderate pressure (e.g., repetitive stress on prominent metatarsal heads during ambulation).

2. How Large Is the Ulcer?

Size of the wound plays a key role in determining duration to wound healing. To simplify wound diameter measurements, one may trace the wound on sterile acetate sheeting and tape this tracing into the chart (Fig. 2.6). The tracing can also be performed on the outer wrapping of an instrument sterilization pack (which would otherwise be discarded). Recently, many centers have begun employing digital photography and computer-driven planimetric wound area calculations. This provides for potentially more consistent, accurate measurements and, ultimately, for comparison of wound healing rates with other centers regionally and beyond. In an evaluation of the reproducibility of wound measurement techniques, Wunderlich and colleagues reported that wound tracing and digital planimetric assessment were by far more reliable than manual measurement of length and width [63].

3. What Does the Base Look Like?

When describing the base of a wound, one may use terms like granular, fibrotic, or necrotic. One may record the presence or absences of any drainage, which may be described as serous or purulent, with a further description of any odor or color.

4. What Do the Margins Look Like?

The margins tell us a lot about the wound. If adequately debrided and off-loaded, they should be well adhered to the surface of the underlying subcuticular structures with a gentle slope toward normal epithelium. However in the inadequately debrided, inadequately off-loaded wound, undermining of the leading edge normally predominates. This is due to the "edge effect" which dictates that an interruption in any matrix (in this case, skin) magnifies both vertical and shear stress on the edges of that interruption. This subsequently causes shearing from the underlying epithelium (making the wound larger by undermining) and increased vertical pressure (making the wound progressively deeper). If appropriately debrided and off-loaded, this effect will be mitigated. Nonetheless, the margins of the wound should be classified as undermining, adherent, macerated, and/or nonviable.

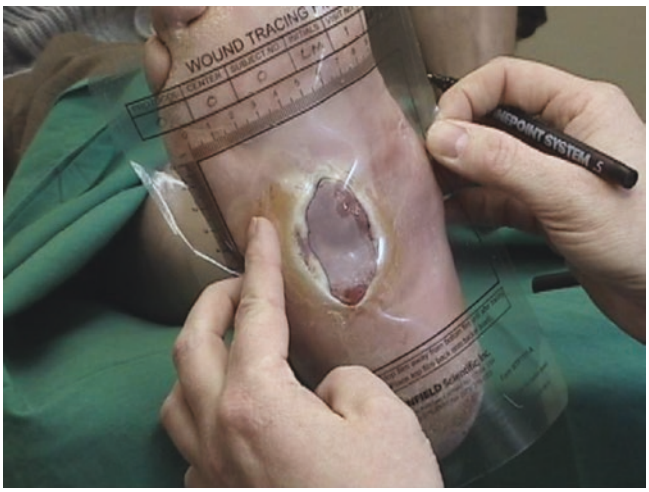

Fig. 2.6 Tracing the wound using sterile acetate sheet. Wound tracing may yield far more reproducible results in measuring wound size than simply length by width measurement

Subsequent to the first questions, which we term "descriptive," come the last three questions which we term "classifiers," These classifiers can then be used to fit a patient into the University of Texas wound classification system (Fig. 2.7). This system has evolved as a significant modification of the Wagner system (Fig. 2.8) to include concomitant depth, infection, and ischemia. While both systems have been shown to be predictive of poor outcomes, the UT system has been shown to be significantly more predictive and complete [64, 65]. Both, however, may be considered useful in a clinical scenario, depending on the preference of the clinician.

5. How Deep Is the Ulceration? Are There Underlying Structures Involved?

These two questions are so closely related that they are combined into one. There is a possible contribution of depth to ulcer healing times [65]. Depth of the wound is the most commonly utilized descriptor in wound classification. Wounds are graded by depth. Grade 0 represents a pre- or post-ulcerative site. Grade 1 ulcers are superficial wounds through the epidermis or epidermis and dermis but do not penetrate to tendon, capsule, or bone. Grade 2 wounds penetrate to tendon or capsule. Grade 3 wounds penetrate to bone or into a joint. We have known for some time that wounds that penetrate to bone are frequently osteomyelitic. Additionally, we have observed that morbid outcomes are intimately associated with progressive wound depth.

		Grade			
		0	1	2	3
Stage	A	**Pre or post-ulcerative lesion completely epithelialized**	**Superficial wound, not involving tendon, capsule, or bone**	**Wound penetrating to tendon or capsule**	**Wound penetrating to bone or joint**
	B	with infection	with infection	with infection	with infection
	C	with ischemia	with ischemia	with ischemia	with ischemia
	D	with infection and ischemia	with infection and ischemia	with infection and ischemia	with infection and ischemia

Fig. 2.7 University of Texas Wound Classification System

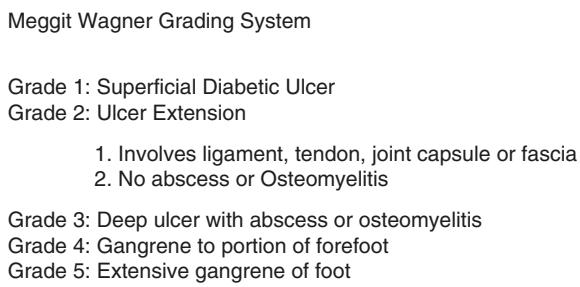

Fig. 2.8 Meggit Wagner grading system

Depth of the wound and involvement of underlying structures may best be appreciated through the probe-to-bone test (PBTB). The probe-to-bone test is performed by inserting a sterile blunt metallic probe into the wound. Since it was first reported in 1995, there have been varying reports about the accuracy of the PBTB [66, 67].

6. Is There Infection?

The definition of bone and soft tissue infection is not an easy one. Cultures, laboratory values, and subjective symptoms are all helpful. However, the diagnosis of an infection's genesis and resolution has been and continues to be a clinical one. While criteria for infection may be something less than clear-cut, there is little question that presence of infection is a prime cause of lower extremity morbidity and frequently eventuates into wet gangrene and subsequent amputation. Therefore, in an effort to facilitate communication and effect consistent results, the foot care team should agree on criteria for this very important risk factor.

7. Is There Ischemia?

As discussed above, identification of ischemia is of utmost importance when evaluating a wound. Ischemic wounds were found to take longer to heal compared to neuropathic wounds without deformities [68]. If pulses are not palpable, or if a wound is sluggish to heal even in the face of appropriate offloading and local wound care, noninvasive vascular studies are warranted followed by a prompt vascular surgery consultation and possible intervention to improve perfusion.

Wagner Ulcer Classifications

Several diabetic classification systems have been reported in the medical literature. This section aims to chronologically review some of the most commonly described classification systems currently used by a variety of practitioners to stage diabetic foot wounds and to discuss outcomes related to their use. One of the most frequently cited diabetic wound classification systems was first described by Meggitt [69] in 1976 and Wagner [70] in 1981. The system is based mainly on wound depth and consists of six wound grades. These include grade 0 (intact skin), grade 1 ("superficial ulcer"), grade 2 (deep ulcer to tendon, bone or joint), grade 3 (deep ulcer with abscess or osteomyelitis), grade 4 ("forefoot gangrene"), and grade 5 ("whole-foot gangrene"). This classification is outlined in Fig. 2.8.

The classification system contains three key descriptors including depth, infection, and ischemia. However, it does not consistently include these important risk factors in every ulcer grade. Infection is included in only one of the six Wagner ulcer grades, and vascular disease is only included in the last two classification grades. The first three grades are concerned only with depth. It is perhaps for this reason that they are the most commonly used, whereas the last three are largely ignored because of their limited clinical use. The descriptors Meggit and Wagner used for ischemia were forefoot and whole foot gangrene. These represent the most severe form of end-stage disease, and therefore cannot help to guide proactive interventional therapy except frank ablation of the affected site. In addition, because gangrene can be caused by infection, it may not always have a vascular origin. Since there are better diagnostic tools to assess and treat PAD, more robust criteria for ischemia will improve diagnosis, interventions, and amputation prevention.

There are several papers that have attempted to validate the Wagner classification system [71, 72]. Calhoun et al. [72] evaluated wounds that were infected and retrospectively assigned Wagner grades to them. They found that when wounds were treated according to what they considered a healthy standard of care, then success, which they defined as eradication of infection and prevention of readmission for 1 year, was frequently achieved despite wound grade. Van Acker et al. [73] found the Wagner classification to have significant association with the duration of healing of the ulcer. Armstrong et al. [65] suggested that patients with Wagner stages 4 and 5 may be grouped together as the two groups did not have separate prognostic value. In addition these patients are often referred directly to a surgeon for amputation and are rarely seen by the diabetic foot team. The system was adapted to combine medical and surgical elements of therapy to monitor the treatment of diabetic foot infection. Unfortunately, in requiring that wounds be infected as an inclusion criterion, it made assessment of this classification problematic, as Wagner wound grades 0–2 classically have no infection descriptor attached to them. In fact, the only mention of infection in this system occurs in grade 3. It is this fact that causes many to customize this system, such that it often takes on distinctly different regional characteristics. This unfortunately limits its usefulness as a standard diabetic foot classification.

Other Ulcer Classifications

In the 1980s and 1990s many authors including Forrest and Gamborg-Nelson [74], Pecoraro and Reiber [75], Arlt and Protze [76], and Knighton [77] proposed their own wound

classifications; however, these systems have not gained universal acceptance. More recent classification systems that have been proposed include the UT classification modification by Van Acker/Peter [73], the PEDIS system by IWGDF members [78], and the S(AD) SAD system proposed by Macfarlane and Jeffcoate [79, 80]. These systems will require validation to gain universal acceptance.

UT Ulcer Classification

The University of Texas Health Science Center in San Antonio (UT) proposed a classification that included depth, infection, and vascular status in 1996 [65, 81]. The classification integrates a system of wound grade and stage to categorize wounds by severity. It is based around two fundamental questions the clinician asks when assessing a wound: (1) How deep is the wound? and (2) Is the wound infected, ischemic, or both? The classification formulates into a matrix with infection and/or ischemia as the vertical axis and depth as the longitudinal axis. This system is illustrated in Fig. 2.7.

Similar to other wound classification systems, the UT system grades wounds by depth. Grade 0 represents a pre- or post-ulcerative site. Grade 1 ulcers are superficial wounds through either the epidermis or the epidermis and dermis but do not penetrate to tendon, capsule, or bone. Grade 2 wounds penetrate to tendon or capsule but the bone and joints are not involved. Grade 3 wounds penetrate to bone or into a joint. Within each wound grade there are four stages: clean wounds (A), nonischemic infected wounds (B), ischemic wounds (C), and infected ischemic wounds (D).

The Grade 0 Wound: Grade 0 wounds are pre-ulcerative areas or previous ulcer sites that are now completely epithelialized after debridement of hyperkeratosis and nonviable tissue. The diagnosis of a grade 0 wound can be made only after removal of any regional hyperkeratosis, as ulcerations may be hidden by overlying calluses. The grade 0-A wound is then a pre-ulcerative area or a completely epithelialized post-ulcerative area. The grade 0-B wound is a 0-A lesion with associated cellulitis. The grade 0-C wound is a 0-A lesion with concomitant regional signs of ischemia. The grade 0-D wound is a 0-B lesion coupled with a working diagnosis of lower extremity ischemia as defined above.

Although lesions that fall into the grade 0 category do not have a break in the epidermis and may not be classically classified as "wounds," the category is important in the identification of sites that are "at risk" for future ulceration and to monitor and prevent re-ulceration of newly healed wounds. Because there is a very high rate or re-ulceration (28–50%), the grade 0 classification allows physicians to follow the progression of wounds over time from healed to re-ulcerated.

The Grade I Wound: Grade I wounds are superficial in nature. They may be either partial or full thickness skin wounds without the involvement of tendon, capsule, or bone. The Grade I-A wound is therefore superficial partial or full thickness wound. The Grade I-B wound is an infected superficial wound. As with any neuropathic lesion, Grade I- B wounds should be examined very carefully. By definition, the Grade I-B wound implies superficial infection without involvement of underlying structures. If the wound shows signs of significant purulence or fluctuance, further exploration to expose a higher grade infection is in order. The Grade I-C wound is I-A plus vascular compromise and the Grade I-D wound is the infected I-B wound with concomitant ischemia.

The Grade II Wound: Grade II wounds probe deeper than the Grade I wounds. Grade II wounds may involve tendon or joint capsule but not bone. The reason for the distinct delineation between wounds that probe to bone and those without bone or joint involvement is because of the high correlation between probing to bone and osteomyelitis [67]. The II-A wound may therefore probe to tendon or joint capsule, but not bone. The II-B wound is II-A plus infection, again the bone and joint are not involved. The Grade II-C wound is II-A plus ischemia, and the Grade II-D wound correspond to II-B plus ischemia.

The Grade III Wound: A wound that probes to bone is categorized as a grade III wound. The modifiers are then added pending the presence of comorbid factor. The III-A wound probes to bone without local or systemic signs of acute infection. The III-B wound probes to bone with signs of acute infection. The III-C wound is identical to III-A with concomitant ischemia. The III-D wound is characterized by active infection, exposed bone, and vascular insufficiency. The criterion for each of the stages is based on clinical and laboratory data. The working diagnosis of lower extremity ischemia may be based on clinical signs and symptoms such as absence of pedal hair, absent pulses, claudication, rest-pain, atrophic integument, dependent rubor or pallor on elevation plus one or more of the noninvasive criteria (transcutaneous oxygen measurements of <40 mm Hg, ankle-brachial index of <0.80, or absolute toe systolic pressure <45 mm Hg) [83–86].

Clean ulcers may be defined as wounds without local or systemic signs of infection. The clinical diagnosis of infection in persons with diabetes is often difficult and defined by narrow, subtle parameters. Wounds with frank purulence and/or two or more of the following local signs may be classified as "infected": warmth, erythema, lymphangitis, lymphadenopathy, edema, pain, and loss of function. Systemic signs of infection may include fever, chills, nausea, vomiting, or generalized malaise [87]. This clinical diagnosis of infection is often obscured by neuropathy and possibly immunopathy [88, 89]. The diagnosis and subsequent treatment of infection may also be assisted by laboratory studies or positive deep tissue cultures or wound based curettage. When osteomyelitis is suspected, bone biopsy with appropriate pathology and culture studies is still the gold standard for diagnosis [87].

Armstrong et al. validated the predictive value of the UT classification system in 1998 [65] and noted a significant overall trend toward an increased prevalence of amputations as wounds increased in both grade (depth) and stage (comorbidity). Patients whose wounds were both infected and ischemic were noted to be almost 90 times more likely to receive a high level amputation compared with patients in a less advanced wound stage, and patients whose wound probed to the underlying bone were over 11 times as likely to receive a high level amputation. Unfortunately the study was retrospective and was not a multicenter trial. In addition, some degree of bias may have been present since the study was carried out by the center that first described the system and the clinicians using it intimately familiar with the system.

Oyibo et al. [90] compared the Wagner classification system with the UT system in a multicenter prospective longitudinal case-control study of 194 patients. The study suggested that both the UT and the Wagner classification system correlated similarly with clinical outcomes. Both systems associated higher grades with a greater likelihood of an ulcer not healing and a greater chance of limb amputation. The trend for grade of the UT classification system was slightly more robust than the trend for grade of the Wagner classification. The inclusion of comorbid factors such as infection and/or ischemia to grade (depth) when classifying an ulcer with the UT system improves description and adds to the predictive power of a wound classification system, especially for ulcers within the same grade level but at a different stage. Based on this, the UT wound classification showed promise as a more practical system.

In conclusion, it is observed that many of the risk factors for neuropathic ulceration, infection, and subsequent amputation may be identified using simple, inexpensive equipment in a primary care setting. A consistent, thoughtful assessment of the diabetic foot is pivotal to identify high-risk patients. Subsequent to the gathering of clinical data through sequential assessment, appropriate classification of the wound becomes paramount in our efforts to document and communicate the level of risk to all members of the health care team caring for the person with diabetes. These simple approaches should improve communication and facilitate amputation prevention.

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Diabetic Neuropathy

3

Solomon Tesfaye and Jing Wu

Abstract

Diabetic neuropathy is a common complication of diabetes and a cause of considerable morbidity and increased mortality. Diabetic neuropathy encompasses several neuropathic syndromes, the commonest of which is diabetic peripheral neuropathy (DPN), the main initiating factor for foot ulceration. Some patients with peripheral neuropathy may experience troublesome neuropathic pain that is difficult to treat. DPN is also associated with autonomic neuropathy that can involve almost all the systems of the body and may have devastating consequences such as sudden death. This chapter looks at the common neuropathic complications of diabetes and their treatment.

Introduction

Diabetic neuropathy is a major complication of diabetes and a cause of considerable morbidity and increased mortality [1]. Diabetic neuropathy is not a single entity but includes several neuropathic syndromes (Fig. 3.1) [2, 3]. In clinical practice, by far the commonest presentation of diabetic neuropathy is chronic distal symmetrical polyneuropathy also known as "diabetic peripheral neuropathy (DPN)". The Toronto Diabetic Neuropathy Expert Group recently defined DPN as "a symmetrical, length-dependent sensorimotor polyneuropathy attributable to metabolic and micro-vessel alterations as a result of chronic hyperglycaemia exposure and cardiovascular risk covariates" [1]. "An abnormality of nerve conduction tests, which is frequently subclinical, appears to be the first objective quantitative indication of the condition [1]. The occurrence of diabetic retinopathy and nephropathy in a given patient strengthen the case that the polyneuropathy is attributable to diabetes" [1].

The neuropathic syndromes depicted in Fig. 3.1 have varied presentations as regards the onset of symptoms, the clinical course and possibly pathogenesis [2]. This chapter will cover all these syndromes although the main focuses will be: (1) DPN, which is the main initiating factor for foot ulceration and a cause of troublesome painful neuropathic symptoms and (2) associated autonomic neuropathy that can involve almost all the systems of the body and may have devastating consequences such as sudden death.

Epidemiology

The epidemiology of DPN shows a lot of variation depending on what tests are employed to detect neuropathy. Where electrophysiology is used the prevalence rates will be in excess of 50% [4], whereas when clinical parameters and/or quantitative sensory testing (QST) are employed both clinic- and population-based studies show surprisingly similar prevalence rates for DPN, affecting around 30% of all diabetic people [5]. The EURODIAB Prospective Complications Study investigated 3250 type 1 patients, from 16 European countries, and found a prevalence rate of 28% for DPN at baseline [6]. The study also showed that over a 7.3-year period, about one-quarter of type 1 diabetic patients developed DPN, age, duration of diabetes and poor glycaemic control being major determinants [7]. The development of DPN was also associated with potentially modifiable cardiovascular risk factors such as hypertension, hyperlipidaemia, obesity and cigarette smoking (Fig. 3.2) [7]. Based on recent epidemiological studies, correlates of DPN include increasing age, increasing duration of diabetes, poor glycaemic control, retinopathy, albuminuria and vascular risk factors [7].

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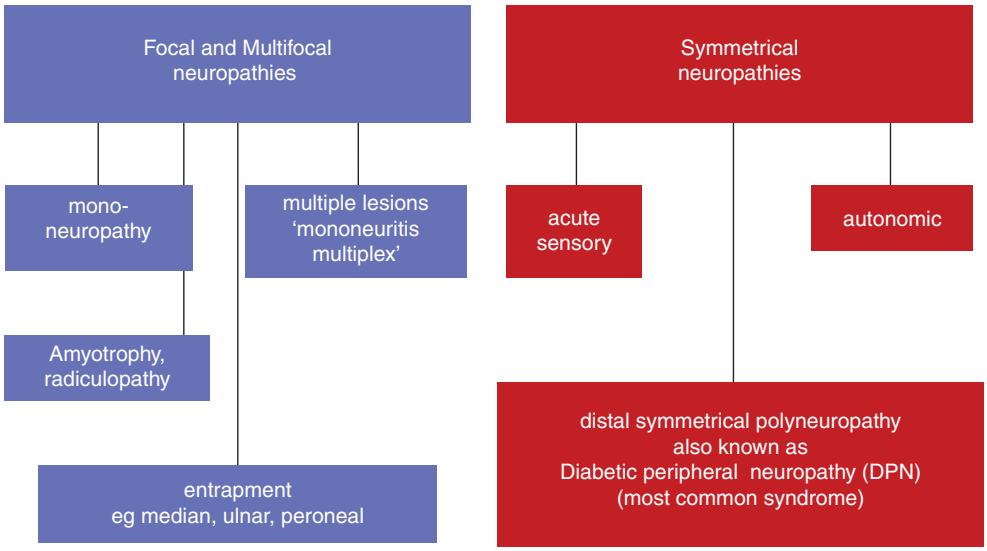

Fig. 3.1 Neuropathic syndromes associated with diabetes mellitus

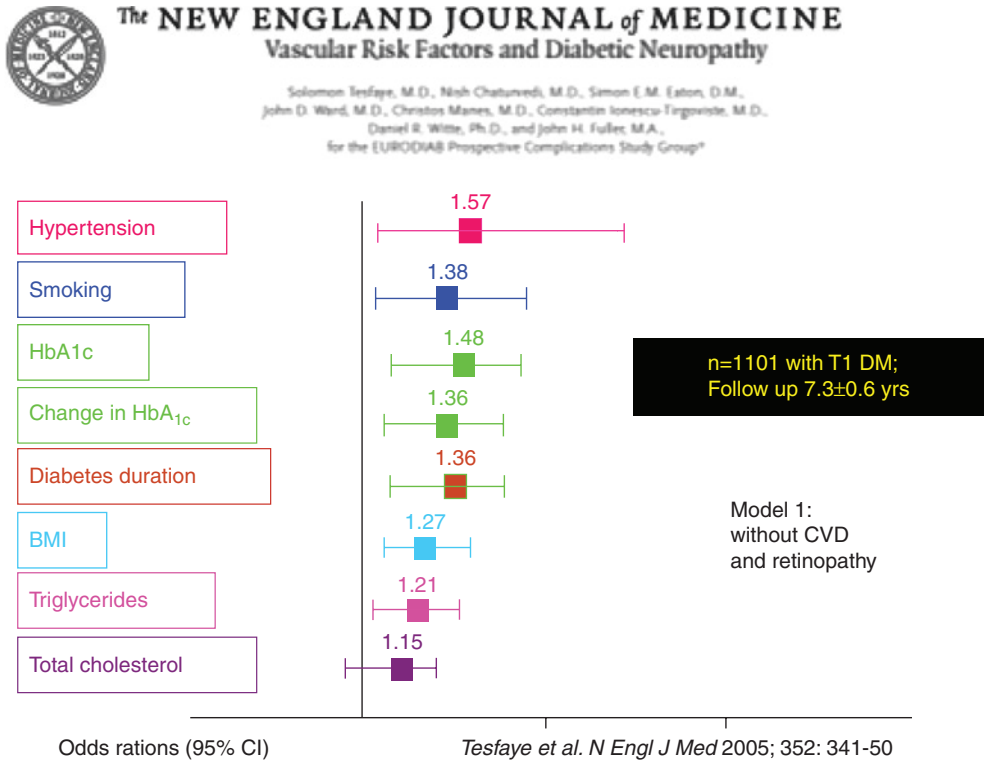

Fig. 3.2 Risk factors for incident DPN in the EURODIAB prospective study [7]

Classification of Diabetic Neuropathy

Classification of the various syndromes of diabetic neuropathy has proved difficult. The variations and overlap in aetiology, clinical features, natural history, and prognosis have meant that most classifications are necessarily oversimplified and none has proved capable of accounting for all these factors. Nevertheless, attempts at classification stimulate thought as to the aetiology of the various syndromes and also assist in the planning of management strategy for the patient.

Figure 3.1 shows a modified clinical classification of diabetic polyneuropathy originally suggested by Thomas [2]. Another method of classifying diabetic neuropathy is by considering whether the clinical involvement is symmetrical or asymmetrical. However, this separation, although useful in identifying distinct entities and perhaps providing clues to the varied aetiologies, is an oversimplification of the truth as there is a great overlapping of the syndromes.

Watkins and Edmonds [8] have suggested a classification for diabetic neuropathy based on the natural history of the

Table 3.1 Classification of diabetic neuropathies by natural history [8]

1. *Progressive neuropathies*. These are associated with increasing duration of diabetes and with other microvascular complications. Sensory disturbance predominates and autonomic involvement is common. The onset is gradual and there is no recovery.
2. *Reversible neuropathies*. These have an acute onset, often occurring at the presentation of diabetes itself, and are not related to the duration of diabetes or other microvascular complications. There is spontaneous recovery of these acute neuropathies.
3. *Pressure palsies*. Although these are not specific to diabetes only, they tend to occur more frequently in diabetic patients than the general population. There is no association with duration of diabetes or other microvascular complications of diabetes.

Table 3.2 Classification of diabetic neuropathies according to the 2017 ADA Position Statement (adapted from [3])

Diabetic neuropathies
A. Diffuse neuropathy
DSPN
• Primarily small-fibre neuropathy
• Primarily large-fibre neuropathy
• Mixed small- and large-fibre neuropathy (most common)
Autonomic
Cardiovascular
• Reduced HRV
• Resting tachycardia
• Orthostatic hypotension
• Sudden death (malignant arrhythmia)
Gastrointestinal
• Diabetic gastroparesis (gastropathy)
• Diabetic enteropathy (diarrhoea)
• Colonic hypomotility (constipation)
Urogenital
• Diabetic cystopathy (neurogenic bladder)
• Erectile dysfunction
• Female sexual dysfunction
Sudomotor dysfunction
• Distal hypohydrosis/anhidrosis
• Gustatory sweating
Hypoglycaemia unawareness
Abnormal pupillary function
B. Mononeuropathy (mononeuritis multiplex) (atypical forms)
Isolated cranial or peripheral nerve (e.g., CM III, ulnar, median, femoral, peroneal)
Mono neuritis multiplex (if confluent may resemble polyneuropathy)
C. Radiculopathy or polyradiculopathy (atypical forms)
Radiculoplexus neuropathy (a.k.a. lumbosacral polyradiculopathy, proximal motor amyotrophy)
Thoracic radiculopathy
Non-diabetic neuropathies common in diabetes
Pressure palsies
Chronic inflammatory demyelinating polyneuropathy
Radiculoplexus neuropathy
Acute painful small-fibre neuropathies (treatment-induced)

various syndromes, which clearly separates them into three distinct groups (Table 3.1).

More recently, in the 2017 Position Statement of the American Diabetes Association, Pop-Busui et al. provide a more detailed classification of the diabetic neuropathies (Table 3.2).

Symmetrical Neuropathies

Diabetic Peripheral Neuropathy (DPN)

Diabetic peripheral neuropathy is the commonest neuropathic syndrome and what is meant in clinical practice by the phrase "diabetic neuropathy" or "diabetic distal symmetrical polyneuropathy (DSP)". There is a "length-related" pattern of sensory loss, with sensory symptoms starting in the toes and then extending to involve the feet and legs in a stocking distribution. In more severe cases, there is often upper limb involvement, with a similar progression proximally starting in the fingers. Although the nerve damage can extend over the entire body including the head and face, this is exceptional. Subclinical neuropathy detectable by autonomic function tests is usually present. However, clinical autonomic neuropathy is less common. As the disease advances, overt motor manifestations such as wasting of the small muscles of the hands and limb weakness become apparent. However, subclinical motor involvement detected by magnetic resonance imaging appears to be common, and thus motor disturbance is clearly part of the functional impairment caused by DPN [9].

The main clinical presentation of DPN is sensory loss which the patient may not be aware of, or may be described as "asleep numbness" or "dead feeling". However, some may experience a progressive build-up of unpleasant sensory symptoms including tingling (paraesthesae or "pins and needles"); burning pain; shooting pains down the legs ("like electric shock"); lancinating pains ("knife like"); contact pain often with daytime clothes and bedclothes (allodynia); pain on walking often described as "walking barefoot on marbles", or "walking barefoot on hot sand or broken glass"; sensations of heat or cold in the feet; persistent achy feeling in the feet and cramp-like sensations in the legs. Occasionally pain can extend above the feet and may involve the whole of the legs, and when this is the case there is usually upper limb involvement also. Table 3.3 summarises the "positive and "negative" symptoms of DPN [10, 11]. There is a large spectrum of severity of these symptoms. Some may have minor complaints such as tingling in one or two toes, others may be affected with the devastating complications such as "the numb diabetic foot", or severe painful neuropathy that does not respond to drug therapy.

Table 3.3 Diagnosis of DPN

Symptoms of DPN	
"Positive" symptoms	
• Persistent burning or dull pain • Paroxysmal electric, shooting, stabbing pain • Dysesthesias (painful paresthesias) • Evoked pain (hyperalgesia, allodynia	
"Negative" symptoms (deficits)	
• Numbness • Hypoalgesia, analgesia • Hypoesthesia, anaesthesia	
Examination: ↓ sensory modalities	

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Table 3.4 Clinical assessment for DPN

History	Signs
• Sensory symptoms	• Inspection (normal or distal wasting, clawing)
• Motor symptoms	• Reflexes (ankle reflex unreliable in the elderly)
• Assessment of disability	• Sensory
	• Vibration
	• Light touch
	• Pinprick (good discriminator in the elderly)
• Exclude other causes of neuropathy	• 10 g Monofilament
	Assess footwear

In DPN there is: ↓ reflexes, vibration, pin prick and pressure sensation
Validated point-of-care devices such as DPN-Check (Neurometrix), SUDOSCAN (Impeto Medical) or Corneal Confocal Microscopy (CCM) may be used

Diabetic neuropathic pain is characteristically more severe at night, and often prevents sleep [12, 13]. Some patients may be in a constant state of tiredness because of sleep deprivation [12]. Others are unable to maintain full employment [14, 15]. Severe painful neuropathy can occasionally cause marked reduction in exercise threshold so as to interfere with daily activities [16]. This is particularly the case when there is an associated disabling, severe postural hypotension due to autonomic involvement. Not surprisingly therefore, depressive and symptoms are not uncommon [17]. Although subclinical autonomic neuropathy is commonly found in patients with DPN [18], symptomatic autonomic neuropathy is uncommon.

It is important to appreciate that many subjects with DPN may not have any of the above symptoms, and their first presentation may be with a foot ulcer [19]. This underpins the need for carefully examining and screening the feet of all diabetic people, in order to identify those at risk of developing foot ulceration. The insensate foot is at risk of developing mechanical and thermal injuries, and patients must therefore be warned about these and given appropriate advice with regard to foot care [19]. A curious feature of the neuropathic foot is that both numbness and pain may occur, the so-called "painful, painless" leg [20]. It is indeed a paradox that the patient with a large foot ulcer may also have severe neuropathic pain. In those with advanced neuropathy, there may be sensory ataxia. The unfortunate sufferer is affected by unsteadiness on walking, and even falls particularly if there is associated visual impairment due to retinopathy.

DPN is usually easily detected by simple clinical examination (Table 3.4) [21]. Shoes and socks should be removed and the feet examined at least annually and more often if neuropathy is present. The most common presenting abnormality is a reduction or absence of vibration sense in the toes. As the disease progresses there is sensory loss in a "stocking" and sometimes in a "glove" distribution involving all modalities. When there is severe sensory loss, proprioception may also be impaired, leading to a positive Romberg's sign. Ankle tendon reflexes are lost (though this may also be lost with old age in non-diabetic people), and with more advanced neuropathy, knee reflexes are often reduced or absent.

Muscle strength is usually normal early during the course of the disease, although mild weakness may be found in toe extensors. However, with progressive disease there is significant generalised muscular wasting, particularly in the small muscles of the hand and feet. The fine movements of fingers would then be affected, and there is difficulty in handling small objects. Wasting of dorsal interossei is however usually due to entrapment of the ulnar nerve at the elbow. The clawing of the toes is believed to be due to unopposed (because of wasting of the small muscles of the foot) pulling of the long extensor and flexor tendons. This scenario results in elevated plantar pressure points at the metatarsal heads that are prone to callus formation and foot ulceration. Deformities such as a bunion can form the focus of ulceration and with more extreme deformities, such as those associated with Charcot arthropathy [22], the risk is further increased. As one of the most common precipitants to foot ulceration is inappropriate footwear, a thorough assessment should also include examination of shoes for poor fit, abnormal wear, and internal pressure areas or foreign bodies.

Autonomic neuropathy affecting the feet can cause a reduction in sweating and consequently dry skin that is likely to crack easily, predisposing the patient to the risk of infection. The "purely" neuropathic foot is also warm due to artero-venous shunting first described by Ward [23]. This results in the distension of foot veins that fail to collapse even when the foot is elevated. It is not unusual to observe a

gangrenous toe in a foot that has bounding arterial pulses, as there is impairment of the nutritive capillary circulation due to arterio-venous shunting. The oxygen tension of the blood in these veins is typically raised [24]. The increasing blood flow brought about by autonomic neuropathy can sometimes result in neuropathic oedema, which is resistant to treatment with diuretics but may occasionally respond to treatment with ephedrine [25].

Recently a number of validated point-of-care devices for the assessment of peripheral neuropathy have immerged. These include DPN-Check (NeuroMetrix, Inc.) [26], SUDOSCAN (Impeto Medical) [27] and Corneal Confocal Microscopy [28].

Autonomic neuropathy affecting the feet can cause a reduction in sweating and consequently dry skin that is likely to crack easily, predisposing the patient to the risk of infection. The neuropathic foot without peripheral vascular disease is also warm due to arterio-venous shunting and distended veins as a result of autonomic neuropathy [23, 24].

Differential Diagnosis of DPN

Before attributing the neuropathy to diabetes other common causes of neuropathy must be excluded. The absence of other complications of diabetes, rapid weight loss, excessive alcohol intake and other atypical features in either the history or clinical examination should direct the physician to search for other causes of neuropathy (Table 3.5).

Table 3.5 Differential diagnosis of DPN

Metabolic
Diabetes
Amyloidosis
Uraemia
Myxoedema
Porphyria
Vitamin deficiency (thiamine, B12, B6, pyridoxine)
Drugs and chemicals
Alcohol
Cytotoxic drugs, e.g. Vincristine
Chlorambucil
Nitrofurantoin
Isoniazid
Neoplastic disorders
Bronchial or gastric carcinoma
Lymphoma
Infective or inflammatory
Leprosy
Guillain-Barre syndrome
Lyme borreliosis
Chronic inflammatory demyelinating polyneuropathy (CIDP)
Polyarteritis nodosa
Genetic
Charcot-Marie-Tooth disease
Hereditary sensory neuropathies

Acute Painful Neuropathies

Acute painful neuropathies are transient neuropathic syndromes characterised by an acute onset of pain (weeks rather than months) in the lower limbs. They are relatively rare compared to chronic DPN. There is often severe neuropathic pain involving both limbs that is distressing to the patient. There are two distinct syndromes, the first of which occurs within the context of poor glycaemic control, and the second with rapid improvement in glycaemic control.

Acute Painful Neuropathy of Poor Glycaemic Control

This occurs usually in type 1 or type 2 diabetic subjects with poor glycaemic control. There is often an associated severe weight loss [29]. Ellenberg coined the description of this condition as "neuropathic cachexia" [30]. Patients typically experience persistent burning pain associated with allodynia (contact pain). The pain is most marked in the feet but often affects the whole of the lower extremities. As in chronic DPN, the pain is typically worse at night although unremitting pain during daytime is also common. The acute-onset distressing pain often results in depression.

In acute painful neuropathies sensory loss is usually surprisingly mild or even absent. There are usually no motor signs, although ankle jerks may be absent. Nerve conduction studies are also usually normal or mildly abnormal. Temperature discrimination threshold (small-fibre function) is however affected more commonly than vibration perception threshold (large-fibre function). Studies are required to investigate if there is loss of intra-epidermal nerve fibre density which may be considered the gold standard in detecting small-fibre neuropathy [31]. There is usually complete resolution of symptoms within 12 months, and weight gain is usual with continued improvement in glycaemic control with the use of insulin.

Acute Painful Neuropathy of Rapid Glycaemic Control (Insulin Neuritis)

The term "insulin neuritis" is a misnomer as the condition can follow rapid improvement in glycaemic control with oral hypoglycaemic agents. The author has therefore recommended that the term "acute painful neuropathy of rapid glycaemic control" be used to describe this condition [32]. Recently, Gibbons and Freeman [33] have recommended the term "treatment-induced neuropathy of diabetes" and reported that this condition is more prevalent than previously thought [33]. The natural history of acute painful neuropathies is an

almost guaranteed improvement [32] in contrast to chronic DPN. The patient presents with burning pain, paraesthesiae, allodynia, often with a nocturnal exacerbation of symptoms; and depression may be a feature. There is no associated weight loss, unlike acute painful neuropathy of poor glycaemic control. Sensory loss is often mild or absent, and there are no motor signs. There is little or no abnormality on nerve conduction studies. Prognosis is good with usually complete resolution of symptoms within 12 months. The management of painful symptoms is as in chronic DPN.

Small-Fibre Neuropathy

The existence "small-fibre neuropathy" as a distinct entity has been advocated by some authorities [32, 34, 35], usually within the context of young type 1 patients and prediabetes [36]. A dominant feature of this syndrome is neuropathic pain, which may be very severe, with relative sparing of large-fibre functions (vibration and proprioception). The pain is described as burning, deep and aching. The sensation of pins and needles (paraesthesae) is also often experienced. Contact hypersensitivity may be present. However, rarely, patients with small-fibre neuropathy may not have neuropathic pain, and some may occasionally have foot ulceration. Autonomic involvement is common, and severely affected patients may be disabled by postural hypotension and/or gastrointestinal symptoms. The syndrome tends to develop within a few years of diabetes (and indeed in prediabetes) as a relatively early complication.

On clinical examination there is little evidence of objective signs of nerve damage, apart from a reduction in pinprick and temperature sensation, which are reduced in a "stocking" and "glove" distribution. There is relative sparing of vibration and position sense (due to relative sparing of the large diameter Aβ fibres). Muscle strength is usually normal and reflexes are also usually normal. However, autonomic function tests are frequently abnormal and affected male patients usually have erectile dysfunction. Electrophysiological tests are usually normal. Quantitative sensory testing (QST) to assess the psychophysical thresholds for cold and warm sensations and skin biopsy with quantification of somatic intra-epidermal nerve fibres (IENF) have been used to determine the damage to small nerve fibres.

Controversy still exists as to whether small-fibre neuropathy is a distinct entity or an earlier manifestation of DPN [34, 35]. Said et al. [34] studied a small series of subjects with this syndrome and showed that small-fibre degeneration predominated morphometrically. Veves et al. [37] found a varying degree of early small-fibre involvement in DPN which was confirmed by detailed sensory and autonomic function tests. It is unclear, therefore, whether this syndrome is in fact distinct or merely represents the early stages of DPN that has been detected by the prominence of early symptoms. The emergence of skin intra-epidermal nerve fibre density as a marker of small-fibre damage may help to clarify the situation [38, 39].

Asymmetrical Neuropathies

Asymmetrical (or focal) neuropathies have a relatively rapid onset, and complete recovery is usual. This contrasts with chronic DPN, where there is usually no improvement in symptoms several years after onset. Unlike DPN their presence is not related to the presence of other diabetic complications. Asymmetrical neuropathies predominantly affect middle-aged/older patients and are more common in men [40]. A high index of suspicion for a non-diabetic cause by conducting careful history/examination in order to identify any associated symptoms/signs is advisable.

Diabetic Amyotrophy

(Proximal Motor Neuropathy, Femoral Neuropathy)

The syndrome of progressive asymmetrical proximal leg weakness and atrophy was first described by Garland [41], who coined the term "diabetic amyotrophy". This condition has also been named as "proximal motor neuropathy" or "femoral neuropathy". The patient presents with severe pain which is felt deep in the thigh, but can sometimes be of burning quality and extend below the knee. The pain is usually continuous and often causes insomnia and depression [42]. Both type 1 and type 2 patients over the age of 50 are affected [41–43]. There is an associated weight loss which can sometimes be very severe, and can raise the possibility of an occult malignancy.

On examination there is profound wasting of the quadriceps with marked weakness in these muscle groups, although hip flexors and hip abductors can also be affected. Thigh adductors, glutei, and hamstring muscles may also be involved. The knee jerk is usually reduced or absent. The profound weakness can lead to difficulty from getting out of a low chair or climbing stairs. Sensory loss is unusual, and if present indicates a coexistent DPN.

Other causes of quadriceps wasting such as nerve root and cauda equina lesions and occult malignancy causing proximal myopathy syndromes (e.g. polymyocytis) should be excluded. MR imaging of the lumbosacral spine is now mandatory in order to exclude focal nerve root entrapment and other pathologies. An erythrocyte sedimentation rate (ESR), an X-ray of the lumbar/sacral spine, a chest X-ray and ultrasound of the abdomen may also be required. Electrophysiological studies may demonstrate increased femoral nerve latency and active denervation of affected muscles [44]. CSF protein is often elevated.

The cause of diabetic proximal motor neuropathy is not fully understood although there is some evidence for ischemic nerve injury from altered immunity often with features suggestive or diagnostic of microvasculitis [45, 46]. Though it tends to occur within the background of DPN [44], the

combination of focal features superimposed on diffuse peripheral neuropathy may suggest vascular damage to the femoral nerve roots, as a cause of this condition [45, 46].

As in DPN there is scarcity of prospective studies that have looked at the natural history of proximal motor neuropathy. Coppack and Watkins [42] have reported that pain usually starts to settle after about 3 months, and usually settles by 1 year, while the knee jerk is restored in 50% of the patients after 2 years. Recurrence on the other side is a rare event. Management is largely symptomatic and supportive. Patients should be encouraged and reassured that this condition is likely to resolve. There is still controversy as to whether the use of insulin therapy influences the natural history of this syndrome. Some patients benefit from physiotherapy that involves extension exercises aimed at strengthening the quadriceps. There is also some evidence that, unlike DPN, immune therapy may be helpful in the treatment of this condition given the pathological substrate ischemic injury from altered immunity [46] and therefore is important to identify early and distinguish from other neuropathies that occur in patient with diabetes [46]. The management of pain in diabetic amyotrophy is similar to that of painful DPN (see below).

Cranial Mononeuropathies

The commonest cranial mononeuropathy is the third cranial nerve palsy. The patient presents with pain in the orbit, or sometimes with a frontal headache [47, 48]. There is typically ptosis and ophthalmoplegia, although the pupil is usually spared [49, 50]. Recovery occurs usually over 6 months. The clinical onset and timescale for recovery, and the focal nature of the lesions on the third cranial nerve, on postmortem studies suggested an ischaemic aetiology [47, 51]. It is important to exclude any other cause of third cranial nerve palsy (aneurysm or tumour) by CT or MR scanning, where the diagnosis is in doubt. Fourth, sixth and seventh cranial nerve palsies have also been described in diabetic subjects, but the association with diabetes is not as strong as that with third cranial nerve palsy.

Thoracoabdominal Neuropathy

(Truncal Radiculopathy)

Diabetic thoracoabdominal neuropathy (truncal radiculopathy) is characterised by an acute onset pain in a dermatomal distribution over the thorax or the abdomen [52]. The pain is usually asymmetrical, and can cause local bulging of the muscle [53]. There may be patchy sensory loss and other causes of nerve root compression should be excluded. Recovery is usually the rule within several months, although symptoms can sometimes persist for a few years. Some patients presenting with abdominal pain have undergone unnecessary investigations such as barium enema, colonoscopy and even laparotomy, when the diagnosis could easily have been made by careful clinical history and examination.

Pressure Palsies

Carpal Tunnel Syndrome

The patient typically has pain and paraesthesia in the hands, which sometimes radiate to the forearm and are particularly marked at night. In severe cases clinical examination may reveal a reduction in sensation in the median territory in the hands, and wasting of the muscle bulk in the thenar eminence. The clinical diagnosis is easily confirmed by median nerve conduction studies and treatment involves the use of splints, steroid injections and surgical decompression at the carpel tunnel in the wrist. There is generally good response to surgery, although painful symptoms may relapse more commonly than in the non-diabetic population.

Ulnar Nerve and Other Isolated Nerve Entrapments

The ulnar nerve is also vulnerable to pressure damage at the elbow resulting in wasting of the dorsal interossei, particularly the first dorsal interosseous. This is easily confirmed by ulnar electrophysiological studies.

Rarely, the patients may present with wrist drop due to radial nerve palsy after prolonged sitting (with pressure over the radial nerve in the back of the arms) while unconscious during hypoglycaemia or asleep after an alcohol binge.

In the lower limbs the common peroneal (lateral popliteal) is the most commonly affected nerve resulting in foot drop. Unfortunately, complete recovery is not usual. The lateral coetaneous nerve of the thigh is occasionally also affected with entrapment neuropathy in diabetes. Phrenic nerve involvement in association with diabetes has also been described.

Pathogenesis of Diabetic Neuropathy

Despite considerable research, the pathogenesis of diabetic neuropathy remains undetermined [54]. Morphometric studies have demonstrated that distal symmetrical neuropathy is characterised by pathological changes including: (1) axonal loss distally, with a “dying back” phenomenon [34], (2) a reduction in myelinated fibre density [55], and (3) focal areas of demyelination on teased fibre preparations [34]. Nerve regenerative activity may also be seen with the emergence of “regenerative clusters” [56], containing groups of myelinated axons and non-myelinated axons sprouts. However, the small and unmyelinated fibres that make up around 80% of all nerve fibres have proved more difficult to assess.

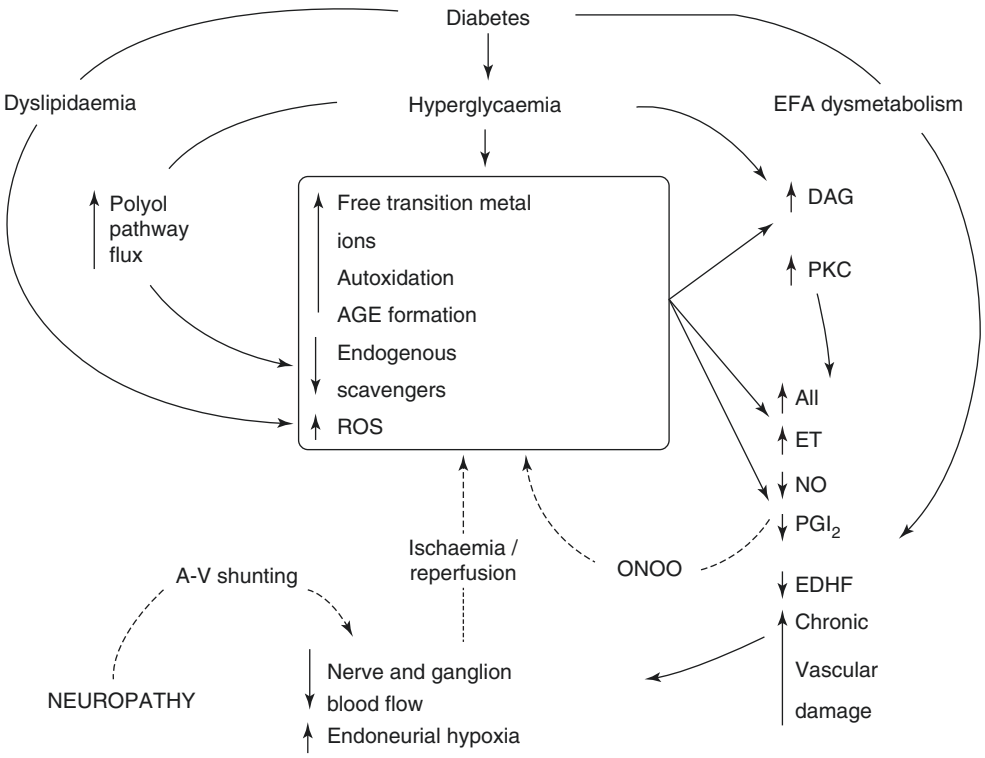

Fig. 3.3 Pathogenesis of DPN. Schematic of the metabolic and vascular interactions that alter neurovascular function in diabetes. *AII* angiotensin 2, *AGE* advanced glycation end product, *A-V* arterio-venous, *DAG* diacylglycerol, *EDHF* endothelium-derived hyperpolarising factor, *EFA* essential fatty acid, *ET* endothelin-1, *NO* nitric oxide, *ONOO-* peroxynitrite, *PGI2* prostacyclin, *PKC* protein kinase, *ROS* reactive oxygen species [57]. (Adapted from figure 6 in Cameron et al. Diabetologia 2001; 44: 1973–88 will need permission)

Figure 3.3 shows current thinking regarding the pathogenesis of diabetic neuropathy [57]. Hyperglycaemia stimulates the production of advanced glycosylated end products, activates protein kinase C, enhances polyol pathway activity and induces a dysregulation of reactive oxygen and nitrogen generating pathways (nitrosative stress) [58]. These processes impair the capacity of the vascular endothelium to produce biologically active nitric oxide (NO), which adversely affects vascular relaxations. Endothelial cells exposed to high extracellular glucose respond by increased mitochondrial superoxide formation [59]. Superoxide combined with NO generated by the endothelial cells (produced by the endothelial isoform of NO synthase) then leads to the formation of peroxynitrite, which attacks various biomolecules in the vascular endothelium [60]. Reactive oxygen and nitrogen species trigger endothelial cell dysfunction through many mechanisms including substrate depletion and uncoupling of endothelial isoform of NO synthase [60]. Another patho-mechanism involves DNA strand breakage and activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP). Poly(ADP-ribose) polymerase (PARP) activation, an important factor in the pathogenesis of diabetes complications, is considered a downstream effector of oxidative-nitrosative stress [60]. However, there is evidence that PARP activation may even precede and contribute to free radical and oxidant-induced injury [61]. PARP-mediated poly(ADP-ribosyl)ation and inhibition of glyceraldehyde-3-phosphate dehydrogenase importantly contributes to the development of diabetic vascular complications: it induces activation of multiple pathways of injury including activation of nuclear factor kappa B, activation of protein kinase C and generation of intracellular advanced glycation end products [60]. Reactive species generation and PARP play key roles in the pathogenesis of "glucose memory" and in the development of injury in endothelial cells exposed to alternating high/low glucose concentrations.

Vascular Factors

The view that micro-vessel disease may be central to the pathogenesis of diabetic neuropathy isn't new [62]. Severe neural microvascular disease has been demonstrated in subjects with of clinical diabetic neuropathy [63]. Several workers have reported basal membrane thickening of endoneurial capillaries, degeneration of pericytes and hypoplasia and swelling of endothelial cells and sometimes vessel closure. The degree of microvascular disease has been correlated with the severity of neuropathy [64].

In vivo studies looking at the exposed sural nerve in human subjects have demonstrated epineural arterio-venous shunting, which appears to result in a "steal" phenomenon diverting blood from the nutritive endoneurial circulation [65]. The consequent impairment of nerve blood flow causes a fall in endoneural oxygen tension [66]. In addition, several other studies provide indirect evidence supporting a vascular aetiology for diabetic neuropathy. Strenuous exercise increases nerve blood flow, and thereby increases nerve conduction velocity by an average of 4 m/s in non-neuropathic diabetic subjects [67]. However, this significant increase in

nerve conduction velocity, with exercise, is absent in neuropathic subjects as the nerve microvasculature is severely diseased [67]. Moreover, there is a strong correlation between nerve conduction velocity and lower limb transcutaneous oxygenation measurements in diabetes; macrovascular disease appears to exacerbate neuropathy and surgical restoration of perfusion improves nerve conduction velocity [68]. A recent epidemiological study has also found a strong correlation between diabetic neuropathy and cardiovascular risk factors, including body weight, hypertension, smoking and hyper-triglyceridemia [7].

In addition to human studies impairment of blood flow has been found to be an early feature in rats with streptozotocin diabetes. Several vasodilators have also been found to enhance nerve blood flow and nerve function in diabetic animals [57]. In human diabetic neuropathy ACE inhibitors have been found to improve nerve function [69, 70]. The presence of severe microvascular changes in subjects with acute painful neuropathy of rapid glycaemic control (insulin neuritis), hitherto thought to be purely metabolic in origin, provides an even more compelling evidence for the importance of microvascular factors in the pathogenesis of DPN [32].

Autonomic Neuropathy

Abnormalities of autonomic function are very common in subjects with long-standing diabetes; however, clinically significant autonomic dysfunction is uncommon. Several systems are affected (Table 3.6). Autonomic neuropathy has a gradual onset and is slowly progressive. The prevalence of diabetic autonomic neuropathy depends on the type of population studied, and a number of tests of autonomic function employed. In the EURODIAB study the prevalence of autonomic neuropathy defined as the presence of two abnormal cardiovascular autonomic function tests was 24%, and the prevalence increased with age, duration of diabetes, glycaemic control and presence of cardiovascular risk factors [71].

Table 3.6 Clinical consequences of autonomic Neuropathy

Cardiac Autonomic Neuropathy
• Sudden death
• Silent ischaemia
• Exercise intolerance
• Orthostatic hypotension
• Foot vein distension/A-V shunting
Gastrointestinal Autonomic Neuropathy
• Gastroparesis
• Diarrhoea or constipation
Bladder hypomotility
• Urinary incontinence/retention
Erectile dysfunction
Gustatory sweating

Cardiovascular Autonomic Neuropathy

Cardiovascular autonomic neuropathy is a serious complication of long-standing diabetes and causes postural hypotension, change in peripheral blood flow and may be a cause of sudden death.

Postural Hypotension

It is now generally accepted that a fall in systolic blood pressure of >20 mm Hg is considered abnormal [71]. Coincidental treatment with tricyclic antidepressants for neuropathic pain and diuretics may exacerbate postural hypotension, the chief symptom of which is dizziness on standing. The symptoms of postural hypotension can be disabling for some patients who may not be able to walk for more than a few minutes. Severely affected patients are prone to unsteadiness and falls. The degree of dizziness does not appear to correlate with the postural drop in blood pressure. There is increased mortality in subjects with postural hypotension, although the reasons for this are not fully clear.

The management of subjects with postural hypotension poses major problems, and for some patients there may not be any satisfactory treatment. Current treatments include: (1) removing any drugs that may result in orthostatic hypotension, such as diuretics, beta blockers and antianginal agents; (2) advising patients to get up from the sitting or lying position slowly, and crossing the legs; (3) increasing sodium intake of up to 10 grams (185 mmol) per day and fluid intake of 2–2.5 l/day (need to be careful in elderly patients with heart failure); (4) the use of custom fitted elastic stockings extending to the waist; (5) treatment with fludrocortisone (starting at 100 μgm per day) while carefully monitoring urea and electrolytes and (6) in severe cases the alpha-1 adrenal receptor agonist, midodrine or occasionally octreotide may be effective [72].

Changes in Peripheral Blood Flow

Autonomic neuropathy can cause arterio-venous shunting, with prominent veins in the neuropathic leg [23]. Leg vein oxygen tension and capillary pressure are increased in the neuropathic leg due to sympathetic denervation [24]. Thus, in the absence of peripheral vascular disease the neuropathic foot is warm, and this may be one of the factors that cause osteopaenia associated with the development of Charcot neuro-arthropathy [22].

Cardiovascular Autonomic Function Tests

Five cardiovascular autonomic function tests are now widely used for the assessment of autonomic function. These tests are non-invasive, and do not require sophisticated equipment. All that is required is an electrocardiogram machine, an aneroid pressure gauge attached to a mouthpiece, a hand grip dynamometer and sphygmomanometer. Table 3.7 shows reference list for cardiovascular autonomic function test [18].

Table 3.7 Reference values for cardiovascular function tests

	Normal	Borderline	Abnormal
Heart rate tests			
Heart rate response to standing up (30:15 ratio)	≥1.04	1.01–1.03	≤1.00
Heart rate response to deep breathing (maximum minus minimum heart rate)	≥15 beats/min	11–14 beats/min	≤10 beats/min
Heart rate response to Valsalva manoeuvre (Valsalva ratio)	≥1.21	–	≤1.20
Blood pressure tests			
Blood pressure response to standing up (fall in systolic BP)	≤10 mmHg	11–29 mmHg	≥30 mmHg
Blood pressure response to sustained handgrip (increase in diastolic BP)	≥16 mmHg	11–15 mmHg	≤10 mmHg

Gastrointestinal Autonomic Neuropathy

Gastroparesis

Autonomic neuropathy can reduce oesophageal motility (dysphagia and heartburn), and cause gastroparesis (reduced gastric emptying, vomiting, swings in blood sugar) [73]. The diagnosis of gastroparesis is often made on clinical grounds by the evaluation of symptoms and sometimes the presence of succussion splash, while barium swallow and follow through, and gastroscopy may reveal a large food residue in the stomach. Gastric motility and emptying studies can sometimes be performed in specialised units, and may help with diagnosis.

Management of diabetic gastroparesis include optimisation of glycaemic control; the use of anti-emetics (metoclopramide and domperidone) and the use of the cholinergic agent which stimulates oesophageal motility (erythromycin which may enhance the activity of the gut peptide, motilin). Gastric electrical stimulation (GES) has recently been introduced as a treatment option in patients with drug refractory gastroparesis to increase the quality of life by alleviating nausea and vomiting frequencies [74]. This service is offered at specialist units.

Severe gastroparesis causing recurrent vomiting is associated with dehydration, swings in blood sugar and weight loss, and is therefore an indication for hospital admission. The patient should be adequately hydrated with intravenous fluids and blood sugar should be stabilised by intravenous insulin, anti-emetics could be given intravenously and if the course of the gastroparesis is prolonged, total parentral nutrition or feeding through a gastrostomy tube may be required.

Autonomic Diarrhoea

The usual presentation is that of diarrhoea which tends to be worse at night, or alternatively some may present with constipation. Both the diarrhoea and constipation respond to conventional treatment. Diarrhoea associated with bacterial overgrowth may respond to treatment with a broad spectrum antibiotic such as erythromycin, tetracycline or ampicillin.

Abnormalities of Bladder Function

Autonomic bladder dysfunction is a rare complication of autonomic neuropathy and may result in hesitancy of micturition, increased frequency of mictruition and in serious cases with urinary retention associated with overflow incontinence. Such a patient is prone to urinary tract infections. Ultrasound scan of the urinary tract and urodynamic studies may be required. Treatments include mechanical methods of bladder emptying by applying supra-pubic pressure, or the use of intermittent self-catheterisation. Anti-cholinesterase drugs such as neostigmine or peridostigmine may be useful. Long-term indwelling catheterisation may be required in some, but this unfortunately predisposes the patient to urinary tract infections and long-term antibiotic prophylaxis may be required.

Gustatory Sweating

Increased sweating usually affecting the face and often brought about by eating (gustatory sweating) can be very embarrassing to patients. Oral anticholinergic agents, including oxybutynin, propantheline and glycopyrrolate, have improved symptoms [75]; however, adverse reactions, including dry mouth, constipation, potential worsening of gastroparesis and confusion, limit their use. Clonidine has also been used with some success but is also limited by side effects including hypotension and dry mouth [75]. Systemic side effects have led to the investigation of non-systemic approaches. Topical glycopyrrolate, a quaternary ammonium antimuscarinic compound, has been shown to significantly decrease the incidence, severity and frequency of sweating with eating and is tolerated well [76, 77]. Botulinum toxin has been used for gustatory sweating, though in most literature it is limited to use in unilateral, surgical-related cases [78].

Management of Painful Diabetic Neuropathy

Painful diabetic neuropathy is common and a cause of much patient distress and disability [13, 79]. Unfortunately, the treatment scenario for painful neuropathy is less than satisfactory as currently available treatment approaches may not completely abolish the pain [80].

The assessment and treatment of painful DSPN should ideally involve a multidisciplinary team (MDT) that may include a diabetologist, a neurologist, the pain clinic team, specialist nurses, podiatrists, psychologists, physiotherapists,

occupational therapists and others. However, in most clinical settings this is not possible and the management falls mainly to the diabetes physician, the primary care physician or neurologist. When treatment is started, a realistic objective would be to achieve around 50% reduction in pain intensity. However, being "realistic" shouldn't be interpreted as less aggressive pursuit of maximum pain relief. Secondary objectives should include restoration or improvement in functional measures, quality of life, sleep and mood. Although it is hoped that improvement in pain will be followed by improvement in functionality, this may not be the case as many of these patients may have other co-morbidities. Moreover, the MDT should discuss potential interventions in addition to pharmacotherapy to help patients optimise function in the presence of residual pain.

A careful history and examination of the patient is essential in order to exclude other possible causes of leg pain such as peripheral vascular disease, prolapsed intervertebral discs, spinal canal stenosis and corda aquina lesions [80]. Unilateral leg pain should arouse a suspicion that the pain may be due to lumbar-sacral nerve root compression. These patients may well need to be investigated with a lumbar-sacral MR imaging. Other causes of peripheral neuropathy such as excessive alcohol intake and B12 deficiency should be excluded. Where pain is the predominant symptom the quality and severity should be assessed. Neuropathic pain can be disabling in some patients and an empathic approach is essential. In general, patients should be allowed to express their symptoms freely without too many interruptions. The psychological support of the patient's painful neuropathy is an important aspect of the overall management of the pain [80].

Glycaemic Control and Lifestyle Modification

There is now little doubt that good blood sugar control prevents/delays the onset of diabetic neuropathy in type 1 diabetes [81]. Similar convincing data is lacking in type 2 diabetes possibly due to: the follow-up of subjects with more advanced DPN or perhaps the use of inappropriate primary end points [82]. However, in prediabetes lifestyle modification with exercise and weight loss appears to halt/reverse neuropathy and relieve neuropathic pain [83, 84]. These findings however require confirmation in larger studies. The view that painful neuropathic symptoms may be improved by improving metabolic control, if necessary with the use of insulin in type 2 diabetes, is not supported by evidence from controlled trials [85]. Nevertheless, current consensus is that the first step in the management of painful neuropathy is an attempt at improving glycaemic control where appropriate. Additionally, as cardiovascular disease is common in patients with DPN [7] and vascular risk factors (hypertriglyceridaemia, hypertension, visceral obesity, etc.) appear to be implicated in the pathogenesis of DPN [7], there is a good rationale for management of vascular risk factors beyond glycaemic control.

Pharmacological Treatment

Tricyclic Compounds

Tricyclic compounds (TCAs) have been used as first-line agents for many years but their use is limited by frequent side effects that may be central or anticholinergic including dry mouth, constipation, sweating, blurred vision, sedation and orthostatic hypotension (with the risk of falls particularly in elderly patients) [86]. For this reason low dose amitriptyline or imipramine 10–25 mg taken at night may be started. Depending upon efficacy and side effects, the dose can gradually be increased to 75 mg/day and on occasions even higher up to 150 mg/day [86]. Higher doses have been associated with an increased risk of sudden cardiac death and caution should be taken in any patient with a history of cardiovascular disease [1].

Serotonin Noradrenaline Reuptake Inhibitors (SNRI)

The Selective Serotonin Noradrenalin Reuptake Inhibitors (SNRI), duloxetine and venlafaxine have been used for the management of painful DSPN [1]. SNRIs relieve pain by increasing synaptic availability of 5-HT and noradrenalin in the descending pathways that inhibit pain impulses. The efficacy of duloxetine in painful DSPN has been investigated in three identical trials and pooled data from these shows that the 60 mg/day and 120 mg/day doses are effective in relieving painful symptoms, starting within a week and lasting the full treatment period of 12 weeks [87]. The main side effects include nausea, somnolence, dizziness, constipation, dry mouth and reduced appetite although these tend to be mild to moderate and are transient. It is advisable to start at 30 mg/day taken with food for the first week and then increase to the standard dose of 60 mg/day. Venlafaxine (150–225 mg/day) is also effective in relieving painful DPN although cardiovascular adverse events limit its use in diabetes [88].

Anticonvulsants

The anticonvulsant gabapentin that binds to the α-2-δ subunit of the calcium channel thereby reducing neurotransmitter release in the hyperexcited neurone, gradually titrated from 100 mg tid to 3600 mg/day, is also effective [89]. More recently, there have been several clinical trials involving pregabalin in painful DPN, and these showed clear efficacy in management of painful DPN [90]. Unlike gabapentin, pregabalin has linear pharmacokinetics and doesn't require a long titration period and is started at 75 mg bd for about a week and increased to 150 mg bd maintenance dose with a maximum dose of 600 mg/day [1]. The side effects include dizziness, somnolence, peripheral oedema, headache and weight gain [90].

Other effective but generally considered second-line drugs [1] for painful DSPN include: other anticonvulsants in particular carbamazepine [1] although it has troublesome side effects including dizziness, somnolence and gait disturbance.

Alpha-lipoic Acid

Infusion of the antioxidant alpha-lipoic acid: at a dose of 600 mg per day orally or intravenously has also been found to be useful in reducing neuropathic pain [91].

Opiates

The opiate derivative tramadol (50–100 mg four times per day) has been found effective in relieving neuropathic pain [92]. Another opiod, oxycodone slow release has also been shown to be effective in the management of neuropathic pain [93].

Topical Capsaicin and Capsaicin Patch

Topical capsaicin works by depleting substance "P" from nerve terminals, and there may be worsening of neuropathic symptoms for the first 2–4 weeks of application. Topical capsaicin (0.075%) applied sparingly 3–4 times per day to the affected area has also been found to relieve neuropathic pain [94]. In patients with painful DPN, capsaicin 8% patch treatment was found to provide modest pain relief and sleep quality improvements versus a placebo patch, similar in magnitude to other treatments with known efficacy, but without systemic side effects or sensory deterioration [95]. Recently, European Commission has granted approval for a label extension for QUTENZA (capsaicin 8% patch) to include the treatment of adult diabetic patients with peripheral neuropathic pain, either alone or in combination with other medicinal products for pain [96]. Although there were initially safety concerns as capsaicin causes small-fibre degeneration, the treatment appears to be safe [97] as the small fibres do regenerate and has been likened to the "pruning of roses" that may explain the mechanism of action [98].

Intravenous Lignocaine

Intravenous lignocaine at a dose of 5 mg per kg body weight with another 30 min with a cardiac monitor in situ has also been found to be effective in relieving neuropathic pain for up to 2 weeks [99]. This form of treatment is useful in subjects that are having severe pain which is not responding to the above agents, although it does necessitate bringing the patient into hospital for a few hours.

Recent Guidelines for Pharmacological Treatment

The European Federation of Neurological Society (EFNS) [100] and The UK National Institute for Health and Clinical Excellence (NICE) [101] proposed that first-line treatments might comprise of TCAs, SNRIs, gabapentin or pregabalin. The American Academy of Neurology recommended that pregabalin is "established as effective and should be offered for relief of painful DPN (Level A evidence)" [102], whereas Venlafaxine, duloxetine, amitriptyline, gabapentin, valproate, opioids and capsaicin were considered to be "probably effective and should be considered for treatment of painful DSPN (Level B evidence)". However, this recommendation was primarily based on achievement of greater than 80% completion rate of clinical trials, which in turn may be influenced by the length of the trials. Finally, the International Consensus Panel on Diabetic Neuropathy recommended TCAs, duloxetine, pregabalin and gabapentin as first-line agents having carefully reviewed all the available literature regarding the pharmacological treatment of painful DPN [1], the final drug choice tailored to the particular patient based on demographic profile and co-morbidities (Table 3.8).

Table 3.8 Pharmacological treatment of painful DPN

• Tricyclic antidepressants (TCAs)
Amitriptyline 25–150 mg/day;
Imipramine 25–150 mg/day
• Serotonine Noradrenaline Reuptake Inhibitors (SNRIs)
Duloxetine 60–120 mg/day
• Anticonvulsants
Gabapentin 300–3600 mg/day
Pregabalin 900–600 mg/day
• Opiates
Tramadol 200–400 mg/day
Oxycodone 20–80 mg/day
Morphine sulphate SR 20–80 mg/day
• Capsaicin
Cream (0.075%) applied sparingly 3–4 times per day)
Patch (8%) treatment must be performed only by a healthcare provider
• IV lignocaine
5 mg/kg given IV over 30 min with ECG monitoring

Comparator and Combination Trials

A major deficiency in the area of the treatment of neuropathic pain in diabetes is the relative lack of comparative or combination studies. Virtually all previous trials have been of active agents against placebo, whereas there is a need for more studies that compare a given drug with an active comparator and indeed lower dose combination treatments [102]. These issues have been highlighted by recent consensus guidelines from international institutions that have emphasised the need for large comparative and combination treatment trials in painful DPN as a matter of priority [102].

Comparator Trials

Bansal et al. compared amitriptyline with pregabalin in painful DSPN in a small, randomised, double-blind, cross-over trial [103]. This study confirmed that whereas there was little difference in efficacy, pregabalin was the preferred drug because of a superior adverse event profile. However, a major drawback of this study was its small size involving 51 patients only with many patients failing to complete the study [103].

Another recent small, cross-over study from the same group as the above study has compared duloxetine with amitriptyline [104]. The study found that both drugs were equally efficacious although of the reported adverse events, dry mouth was more common with amitriptyline than duloxetine

(55 vs. 24%; P < 0.01). Numerically more patients preferred duloxetine although this was not statistically significant (48 vs. 36%; P = 0.18).

The lack of direct comparator studies led to an indirect comparison of the efficacy and tolerability of duloxetine with that of pregabalin and gabapentin in participants with painful DSPN, using placebo as a common comparator [105]. Efficacy criteria were: reduction in 24-h pain severity for all three treatments, and treatment response rate (≥50% pain reduction) and overall health improvement (as measured on the Patient Global Impression of Improvement/Change questionnaire) for duloxetine and pregabalin only. Indirect comparison between duloxetine and gabapentin found no statistically significant differences. Comparing duloxetine with pregabalin, the authors found significant differences in overall health improvement, favouring pregabalin, and in dizziness, favouring duloxetine. There was no significant difference in 24-h pain severity between duloxetine and pregabalin [105].

Combination Trials

Gilron et al. studied nortriptyline and gabapentin either in combination or alone in a randomised trial and confirmed that when given together, they were more efficacious than either drug given alone [106]. In another cross-over study by the same group, low dose combination therapy with gabapentin and morphine was significantly more effective than higher doses of either [107].

The COMBO-DN study [108] is the largest combination trial in painful DSPN and assessed whether combining standard doses of duloxetine and pregabalin is superior to increasing each drug to its maximum recommended dose in patients with incomplete pain relief. Patients with painful DSPN with a daily pain score of at least 4 (scale 0–10) were randomly assigned in a 1:1:1:1 ratio to 1 of 4 groups. For the 8-week *Initial Treatment* period patients in groups 1 and 2 were treated with 60 mg duloxetine/day; patients in groups 3 and 4 received 300 mg pregabalin/day. Thereafter, only non-responders (<30% improvement in pain relief) received double-blind treatment for further 8 weeks of the *Combination vs. high dose Monotherapy Treatment* period with duloxetine 120 mg/day for group 1, duloxetine 60 mg/day + pregabalin 300 mg/day for groups 2 and 3, and pregabalin 600 mg/day for group 4. The primary outcome was change in the Brief Pain Inventory 24-h average pain during *Combination vs. high dose Monotherapy Treatment* period between (groups 1 and 4 pooled—i.e. high dose Monotherapy) with combination therapy (groups 2 and 3 pooled).

804 patients were evaluated in the Initial and 339 in the *Combination vs. high dose Monotherapy Treatment* period, respectively. The difference between Combination and Monotherapy in the mean change of BPI-MSF average pain during *Combination vs. high dose Monotherapy Treatment* period was not statistically significant (Combination: −2.35; Monotherapy:−2.16; $p = 0.37$). Proportions of patients with treatment emergent adverse events were however similar: 36.7% (Combination) and 33.5% (Monotherapy). As a secondary end point the COMBO-DN study also compared the efficacy of standard doses of duloxetine and pregabalin as initial treatment for painful DSPN, and duloxetine was found to have superior efficacy compared to pregabalin, without any safety findings of concern. At the end of the *Combination vs. high dose Monotherapy Treatment* period, although the groups are no longer randomised, 50% pain relief was found in 46.9% of subjects on 600 mg/day pregabalin compared to 28.4% on 120 mg/day of duloxetine.

Taken together, even though the primary end point was not met, the COMBO-DN study demonstrated that at standard doses duloxetine has better efficacy than pregabalin as an initial treatment for painful DSPN, without any safety findings of concern. However, pregabalin catches up with duloxetine in terms of efficacy as the doses are increased to maximum.

Management of Disabling Painful Neuropathy Not Responding to Pharmacological Treatment

Neuropathic pain can sometimes be extremely severe, interfering significantly with patients' sleep and daily activities. Unfortunately some patients are not helped by conventional pharmacological treatment. Such patients may respond to electrical spinal cord stimulation which relieves both background and peak neuropathic pain [109]. This form of treatment is particularly advantageous, as the patient does not have to take any other pain-relieving medications, with all their side effects.

Tailoring Treatment to Individual Requirements

The initial selection of a particular first-line treatment will be influenced by the assessment of contraindications, evaluation of co-morbidities (including sleep disturbance, mood disorders and other chronic medical/diabetic complications) and cost[65]. For example, in diabetic patients with a history of heart disease, elderly patients on other concomitant medications such as diuretics and anti-hypertensives and patients with co-morbid orthostatic hypotension TCAs have relative contraindications. In patients with liver disease, duloxetine should not be prescribed, and in those with peripheral oedema, pregabalin or gabapentin should be avoided. Moreover, although pharmaceutical companies may recommend a particular starting dose for their drugs based on their clinical trials, one has to appreciate that the clinical practice scenario is different from clinical trial scenario as many elderly patients with multiple co-morbidities would have been excluded from trials. Therefore, treatment has to be individualised to take patient co-morbidities including occupation, renal impairment, etc. into account and caution advised to start at lower than recommended doses and titrating gradually.

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Diagnosis of Peripheral Artery Disease in the Diabetic Patient

4

Sarah E. Deery and Raul J. Guzman

Abstract

Peripheral artery disease (PAD) affects over 200 million patients globally. Patients with diabetes have a twofold higher risk of developing PAD, and with this comes a higher rate of foot ulceration and amputation. The prompt diagnosis and treatment of PAD in diabetic foot ulcer patients is of paramount importance to wound healing and limb preservation. This chapter will outline key features of the initial evaluation for PAD in patients with diabetes. It will also describe the various noninvasive diagnostic modalities available for use, along with their advantages and disadvantages.

Clinical Features of PAD in Diabetes

Peripheral artery disease (PAD) is the partial or complete obstruction by atherosclerosis of arteries supplying the lower extremities. It is estimated to affect over 200 million people globally with a spectrum of presentation ranging from asymptomatic to severe ischemia [1]. Diabetes is a well-known risk factor for PAD [2, 3]. It increases the risk of developing lower extremity atherosclerosis over twofold, and there is a 28% increase for every 1% increase in HbA_{1c} [3, 4]. More importantly, diabetic patients with PAD have a fivefold higher rate of amputation [5].

It is notable that pedal ischemia in patients with diabetes is complex, with multiple overlapping pathologies that can synergistically contribute to decreased distal perfusion. Foot blood flow alterations in diabetes are most commonly attributed to both atherosclerotic occlusive disease involving medium-sized arteries and a less-well-understood microcirculatory dysfunction. These changes are believed to affect blood flow at the arteriolar and capillary levels [6]. It has long been postulated that the microcirculatory impairment seen in patients with diabetes can lead to decreased tissue perfusion resulting in increased susceptibility to even moderate levels of ischemia. Therefore, the identification and treatment of PAD is especially important in this high-risk population [6]. A detailed discussion of the microcirculatory defects seen in the diabetic foot is found in Chap. 10 of this book.

While patients with diabetes may present with atherosclerosis in any peripheral artery, its characteristic occlusive lesions are most commonly found in the infrageniculate, tibial arteries of the calf. A patient may thus present with a wide range of symptoms depending on the specific segmental distribution of disease. For example, diabetic patients with a history of tobacco use may initially present with hip and thigh claudication related to aortoiliac occlusive disease. Patients may also present with calf claudication related to occlusion of the superficial femoral artery. However, patients with isolated tibial artery occlusion may remain asymptomatic until they suffer a minor trauma to the foot. Such an insult is commonly cited as the initial factor in the pathway to pedal ulceration initially proposed by Pecoraro, Reiber, and colleagues [7, 8]. For this reason, a high level of suspicion for the presence of PAD must be maintained in all diabetic patients, and particularly in those with foot ulcers.

PAD Screening in the Non-ulcer Patient

Assessment of the diabetic patient for PAD when there is no pedal pathology may proceed routinely, in distinction to that in the patient with an ulcer. All patients with diabetes should undergo inspection of the foot for signs of ischemia on an annual basis. Evidence of decreased pedal perfusion may be discerned from various physical findings including absence of hair growth, dry, cool, or fissured skin, thickened nails,

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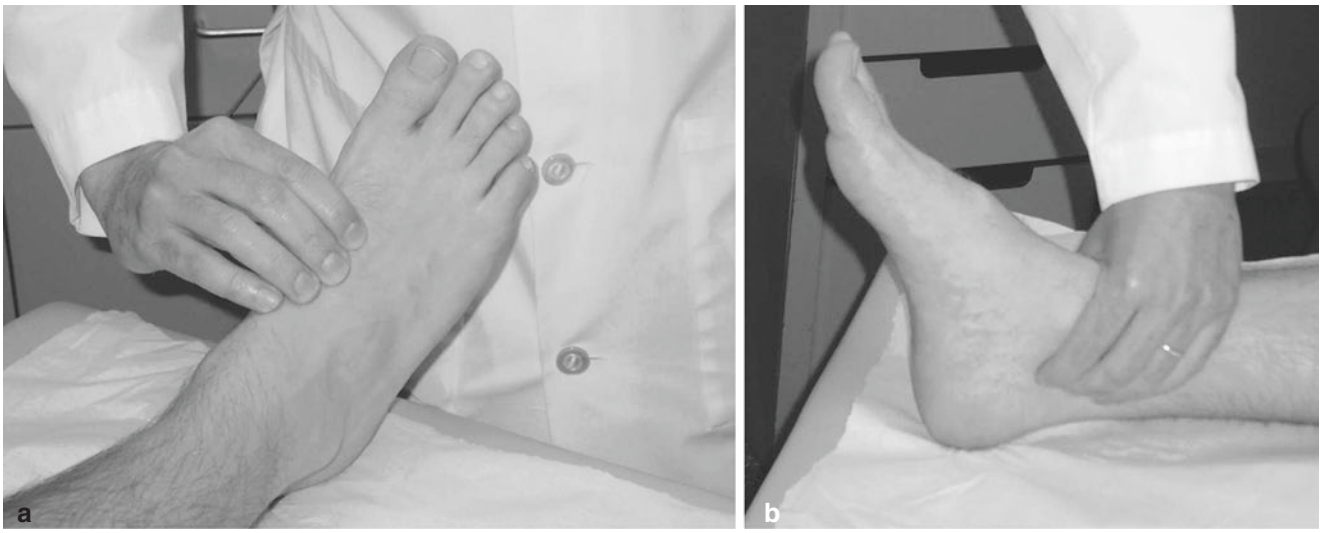

Fig. 4.1 Palpation of the dorsalis pedis (**a**) and posterior tibial (**b**) artery pulses. Note that the examiner's left hand is used to palpate the right posterior tibial artery pulse, allowing the hand to cradle over the top of the ankle

elevation pallor, and dependent rubor [9, 10]. Ankle pulse assessment should also be performed as part of a patient's annual risk assessment. This involves palpation of the dorsalis pedis (DP) and posterior tibial (PT) pulses in each leg. The DP pulse is found between the 1st and 2nd metatarsals, just lateral to the extensor halluces longus tendon, and should be identified with the fingers draped over the dorsum of the foot. The PT artery pulse will be identified just behind the medial malleolus, about halfway between the malleolus and the Achilles tendon (Fig. 4.1). For patients with palpable ankle pulses and without evidence of pedal pathology, no additional testing is required.

For patients over 50, an American Diabetes Association consensus panel on PAD has recommended baseline assessment of ankle-brachial indices (ABIs), with repeat studies performed every 5 years for those without abnormalities [11]. The ankle-brachial index (ABI) is a frequently used measure of peripheral artery disease. It is calculated as a ratio of pressures in the ankles to the brachial arteries. This test has the advantage of convenience and low cost and can be performed with a standard blood pressure cuff and handheld Doppler. The test begins by determining the highest brachial pressure between the right and left arms. After applying the blood pressure cuff to the upper arm, the Doppler probe is positioned over the brachial artery at the antecubital fossa. The cuff is inflated until the Doppler signal cannot be heard. It is then deflated until the Doppler signal returns and this is noted as the brachial pressure in a manner analogous to blood pressure measurement using a stethoscope. The process is then repeated in the contralateral arm, and the highest brachial pressure between the right and left arms is used in the ABI calculation for both legs. Attention is then turned to the ankles where using a Doppler probe sequentially on the PT and DP arteries, the pressure in the cuff is increased to occlude the arteries and released until the Doppler signal returns. The pressure at which the signal returns is noted for each ankle artery and the highest of the DP or PT is used in the calculation for each leg. The process is then repeated for the contralateral extremity. One ABI is calculated for each leg (Fig. 4.2).

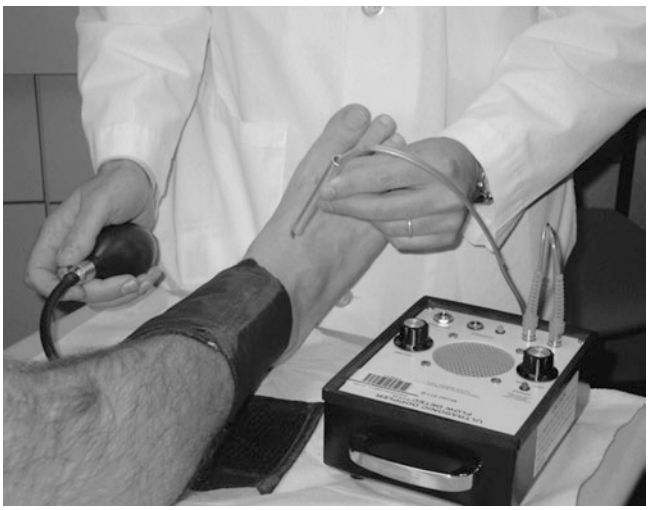

Fig. 4.2 Measurement of the ankle pressure

While there is some debate on the exact ranges that separate ischemia classes, an ABI between 1.1 and 1.3 is typically considered normal and can be used to exclude patients with significant arterial disease when they are asymptomatic. An ABI between 0.4 and 0.9 suggests moderate ischemia, and when it is <0.4, it generally signifies severe ischemia. Additionally, an

ABI greater than 1.3 suggests noncompressible arteries and is typically recorded as "nc." As a screening tool, ABIs can be used as a prognostic marker of cardiovascular risk, and when <0.9 it has been associated with 67% increased risk of cardiac death [12]. Baseline ABI values are useful in patients who subsequently develop foot ulcers so that the time course of circulatory changes can be determined [13]. Significant limitations of ABI testing exist, however, and for this reason, reliance on them as singular tool to assess pedal perfusion should be reserved only for those patients without active ulceration or other ischemic symptoms.

Diagnosis of PAD in the Ulcer Patient

The importance of early arterial assessment in a diabetic foot ulcer patient cannot be overstated. The presence of ischemia increases the risk of amputation 5- to 10-fold [14–16], and delay in treatment of arterial disease can result in further tissue loss. The initial evaluation consists of a comprehensive history and physical exam. Selective noninvasive arterial studies are then performed to objectively evaluate the vascular status of the foot. When the presence of ischemia is established, the patient may then undergo further anatomic imaging and revascularization as discussed in Chap. 20.

The Vascular History and Physical Examination

Patients with diabetes presenting with a foot ulcer should undergo evaluation for underlying arterial insufficiency as part of the initial assessment. Past symptoms of claudication, ischemic rest pain, or prior assessments for vascular disease should be sought. Notably, patients with diabetes may not experience PAD symptoms before developing ulcers either because their occlusive disease is localized to the calf or because neuropathy may mask traditional PAD symptoms. A prior history of foot ulcers, amputations, and other treatments for PAD including endovascular or open bypass procedures should be obtained. Additionally, factors that may assist with clinical decision-making should be noted such as the patient's baseline ambulatory status, goals of care, and cardiovascular perioperative risk.

Physical examination should focus on a description of the ulcer, signs of ischemia, and a thorough pulse exam. Ischemic ulcers are more likely to be present on the most distal parts of the toes whereas those related to neuropathy most often occur on weight-bearing areas such as the plantar surface of the metatarsal heads or over bony deformities. However, at least 30% of patients with neuropathic ulcers have evidence of pedal ischemia, and therefore ulcer location should not be used to exclude PAD [17, 18]. Other suggestions of impaired lower extremity perfusion such as those described in the non-ulcer patient are also sought including skin fissuring, dystrophic toenails, and pallor with elevation or dependent rubor.

A formal pulse assessment should be documented in all patients with diabetic foot disease. The exam begins at the inguinal region with palpation of the femoral pulse that can be identified two fingerbreadths lateral to the pubic tubercle, just below the inguinal ligament. A diminished or absent femoral pulse suggests aortoiliac disease and is usually found in patients with a history of tobacco use. The popliteal pulse is best palpated with the patient in the supine position and with a slightly flexed knee. With the clinician's thumbs placed on the tibial plateau, both hands are wrapped around the leg and the fingers of each are used to feel the pulse in the popliteal space. The pulse exam concludes with assessment of the DP and PT pulses as above. A typical pattern involves the presence of a palpable popliteal pulse but absent ankle pulses suggesting diabetic tibial artery occlusive disease; however, more proximal disease may be identified.

Evaluation of the arterial system in a diabetic foot ulcer patient, however, remains problematic because it is difficult to assess the adequacy of perfusion by physical exam and ABI alone. While the presence of palpable ankle pulses may serve as an adequate screening approach for PAD, several studies have documented the inadequacy of this approach for those with ulcers [19, 20]. Pulse assessment is not reproducible between different individuals, and it is possible to palpate a pulse in a foot with an ABI as low as 0.5 [21]. While ABIs are convenient and easily performed by nonspecialized personnel, significant issues limit their use in a patient with an active ulcer. Patients with diabetes commonly have medial artery calcification and this may lead to an inability to occlude ankle arteries even at pressures significantly above systolic leading to falsely elevated values greater than 1.4. More concerning is the possibility that patients with partially compressible arteries can have ABIs in the normal range even in the presence of severe ischemia. Additionally, peripheral edema and inappropriately sized or applied cuffs may lead to inaccurate results. For these reasons, evaluation of the diabetic ulcer patient will most commonly require use of a certified vascular laboratory with appropriately trained technologists to perform the study and a vascular specialist to provide interpretation.

Noninvasive Arterial Testing

Multiple noninvasive arterial studies are available to the provider of diabetic foot care. Decisions regarding the most appropriate test are based on test availability, familiarity with the technology, and ability to interpret the findings. Vascular specialists will often use a combination of noninvasive studies to assess pedal perfusion.

Segmental Doppler Pressures with ABIs

Segmental pressures can be obtained by placing cuffs at multiple levels along the leg, usually the thigh, calf, and ankle levels. A Doppler probe is then placed on the ankle artery with the best signal. The cuffs are sequentially inflated to yield a pressure at each level. Typically, pressures obtained from the more proximal segments are higher than those at the ankle. The final pressures obtained with the blood pressure cuff at the ankle are used to calculate the ABI. A drop in pressure greater than 20 mm Hg between any two levels suggests arterial disease in the intervening segment [22].

Doppler Waveform Analysis

Normal peripheral arteries have a triphasic waveform with a brisk upstroke of forward flow during systole due to myocardial contraction, followed by a reversal of flow during early diastole, and a small forward component in late diastole [23]. Waveform evaluation at various levels can provide evidence of PAD. With arterial obstruction, one first sees widening of the waveform, then dampening of the amplitude, and eventually transition to a biphasic then monophasic signal (Fig. 4.3). Doppler waveforms are minimally affected by medial calcification and can occasionally be used along with ABIs as the final arterial study in patients with foot ulcers. A foot ulcer patient with normal, triphasic ankle waveforms and an ABI 1.1–1.3 is unlikely to have significant occlusive disease above the ankle. However, the study is operator dependent and not quantitative. Furthermore, triphasic ankle waveforms do not preclude pedal occlusive disease.

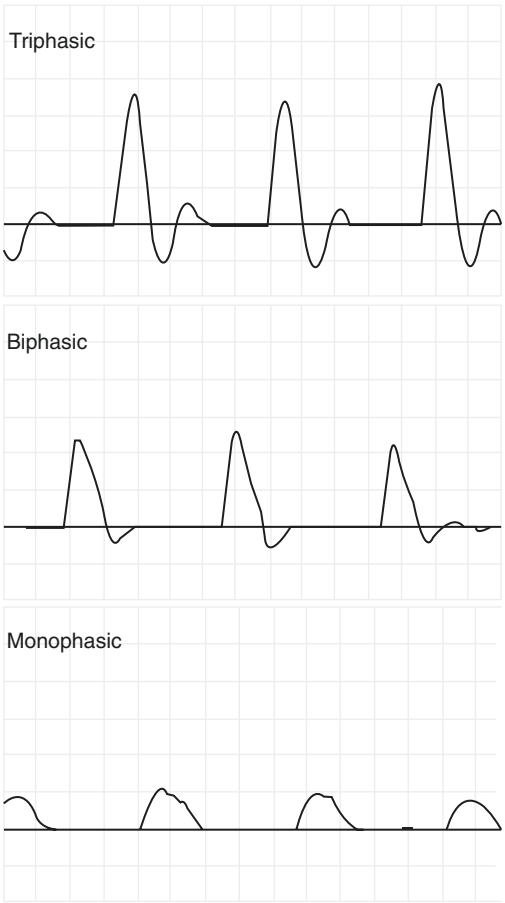

Fig. 4.3 Arterial Doppler waveforms. A triphasic waveform is consistent with normal arterial flow. Biphasic and monophasic waveforms are consistent with moderate and severe occlusive disease

Pulsed Volume Recordings (PVRs)

Plethysmography is the measurement of volume changes in a body part. PVRs are a form of volume plethysmography that work by measuring the pulsatile volume changes that occur in the extremities with each heartbeat (Fig. 4.4). To obtain PVRs, a pneumatic cuff is placed around each level of the limb (high thigh, low thigh, calf, ankle, metatarsal) and inflated to a preset pressure of 60 ± 5 mm Hg [24, 25]. Changes in volume detected by the cuff are shown as oscillating waveforms. While there are similarities between PVR and Doppler-derived waveforms, certain characteristics can be used to distinguish them. A normal PVR waveform first displays a brisk rise during systole, a sharp systolic peak, a dicrotic notch (not seen in Doppler waveforms), and a rapid downslope to baseline. Also unlike Doppler waveforms, there is no portion of the curve below the baseline. With peripheral artery occlusion, the dicrotic notch is lost, the downslope becomes delayed, and the waveform amplitude becomes reduced. In severe disease, all phasic components are ultimately lost. Because PVRs measure changes in volume rather than pressure, they are minimally affected by noncompressible vessels. Although efforts to quantify PVRs have been undertaken [26], the test is essentially qualitative, and can underestimate the severity of proximal arterial disease due to the presence of collaterals. The temperature of the room may affect metatarsal waveforms, and as with Doppler, obesity and peripheral edema may affect the results.

Toe Pressures

The medial calcification of tibial vessels in patients with diabetes may spare vessels in the toe. For this reason, toe pressures and toe-brachial indices (TBI) are often used in patients with diabetes. A photoplethysmography (PPG) probe is used to detect changes in skin capillary blood flow (Fig. 4.5). The great toe is most commonly used for TBI measurements. A special toe cuff is wrapped around the base of the toe and used in a manner analogous to ankle pressure assessments

for ABIs. After a stable baseline PPG tracing is obtained, the toe cuff is inflated to a pressure sufficient to stop pulsatility. It is then deflated until the PPG waveform returns. The pressure at which PPG pulsatility returns is noted and documented as the absolute toe pressure. This pressure may also be used for calculation of the toe brachial index (TBI) similar to use of the ABIs. In a patient with diabetes, a toe pressure above 55 mm Hg is generally associated with adequate perfusion for healing [27], although some suggest that toe pressures above 30 mm Hg may be adequate [28]. However, the study may not be useful in the case of toe amputations, large ulcers, or dressings. Furthermore, the PPG tracing may be affected by any vasoconstrictive states, including cold temperatures and medications.

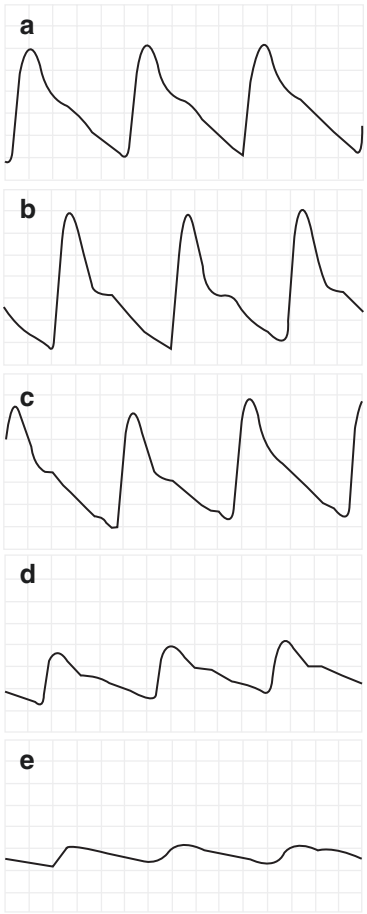

Fig. 4.4 Pulse volume recordings (PVRs) in patient with tibial occlusive disease obtained at the proximal and distal thigh (A, B), proximal calf (C), ankle (D), and metatarsal (E) levels. Note normal diacritic notch in A–C. Dampened waveforms with decreased amplitude at metatarsal level

Transcutaneous Oxygen Tension ($TcPO_2$)

Transcutaneous oxygen tension measurements reflect the metabolic state of the underlying skin. The test involves placement of a probe with a sensitized electrode on the dorsum of the proximal foot. It quantifies the transfer of oxygen molecules to the skin surface. The local tissue is heated to 42–45 °C for optimal blood flow and diffusion of oxygen. Following an equilibration period, the local resting oxygen tension of the skin is recorded in mm Hg. A value of less than 20 mm Hg is associated with severe ischemia, while values greater than 60 mm Hg are interpreted as normal. A meta-analysis involving 31 studies concluded that a TcPO2 value below 40 mm Hg was associated with a 24% increased risk of healing complications, however, the added value of this technology over standard clinical data could not be determined [29]. Unfortunately, the method is relatively insensitive to mild or moderate degrees of PAD because the oxygen supplied to the skin is often greater than the demand. This test should be interpreted with caution, as multiple factors can affect the results, including skin and body temperature, age, and oxygen diffusion through tissues. Other variables often seen in patients with diabetes, particularly those with a diabetic foot, including obesity, peripheral edema, and cellulitis, can also influence the findings [30].

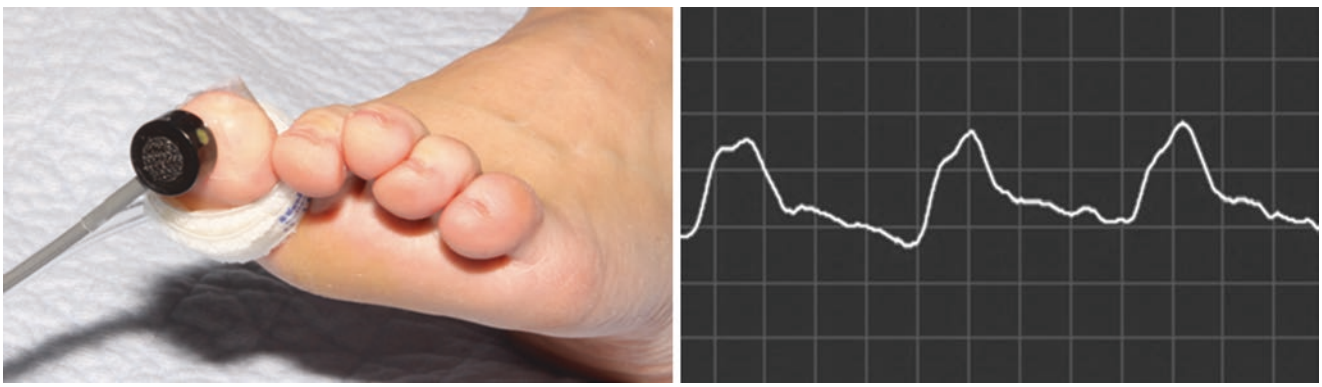

Fig. 4.5 Toe pressure measurement. Photoplethysmography (PPG) probe is attached to distal left great toe with tape. Proximal cuff is inflated past the point that PPG waveform is abolished. Pressure at which waveform returns is recorded as toe pressure in mm Hg

Laser Doppler Perfusion

The Laser Doppler flow technique allows for an assessment of skin perfusion in the target tissue. Laser light is transmitted to the tissue via a fiber-optic probe and the returning light is processed. The relative number and velocity of blood cells in the tissue are calculated and used as an estimate of perfusion. One study showed good accuracy and positive predictive value for wound healing with laser Doppler flowmetry >50 mv, and 100% specificity for wound healing with measurements >125 mv [31]. Values <35 mv have a relatively high negative predictive value for wound healing. However, the large range of values between these two cutoff points, where many diabetic patients reside, is considered a gray zone and may not be useful for establishing the presence of ischemia.

Skin Perfusion Pressure (SPP)

An effective measure of distal perfusion at the site of ulceration is the skin perfusion pressure that can be measured using the laser Doppler or PPG probe. After warming the involved extremity to 42 °C with a heating pad, the tester applies the laser Doppler probe with cuff to the area under investigation, usually on the dorsum of the affected foot. The cuff is inflated to 5–10 mmHg and a baseline laser Doppler output is recorded. The cuff is then inflated to at least 20 mmHg over the systolic blood pressure and deflated slowly until the laser Doppler output increases for consecutive pressure values. The pressure before the first increase is the SPP. Alternatively, skin perfusion pressure can be measured using photoplethysmography along with a blood pressure cuff as above, although the results using PPG may not be as reliable [32].

Skin perfusion pressure, whether measured by laser Doppler or PPG, can be used to assess perfusion and is unaffected by medial artery calcification. Because it can be measured anywhere on the limb, it can be used to assess peri-ulcer tissues. Values >30 mm Hg are predictive of wound healing, whereas values <30 mm Hg have been associated with increased risk of amputation [33–35].

Arterial Duplex Ultrasound

Duplex scanning employs the dual modalities of B-mode (gray scale) imaging and pulsed wave Doppler spectral frequency analysis. In addition, most Duplex scanners are actually "triplex," with the third modality being color-flow imaging. The B-mode, or brightness mode, image is displayed as gray-scale pixels and reflects the amplitude and position of returning ultrasound echoes, which allows for vessel localization. Optimal arterial anatomic imaging occurs with transducer beam directed perpendicular to the vessel wall, and this allows the operator to measure vessel diameter, identify intima-media thickening, and assess atherosclerotic plaque composition. Pulsed wave Doppler spectral analysis is useful in quantifying the degree of stenosis by identifying the peak systolic velocity or evaluating the waveforms. Color flow is used to distinguish between direction of flow towards or away from the transducer but can also be helpful in establishing points of turbulence associated with stenoses. The primary advantage of Duplex ultrasound is that it can be used for anatomic assessment of arteries and to determine the distribution of occlusive lesions. The use for routine peripheral arterial disease evaluation is more limited given the wide variation in normal lower extremity arterial distribution and the association between inconclusive studies and tibial vessel medial calcification, as is often seen in patients with diabetes [36]. However, the technique is operator-dependent, and can be time consuming. Like other techniques, the results are also influenced by medial arterial calcification, obesity, and peripheral edema.

Noninvasive Axial Imaging by CTA and MRA

The initial assessment and diagnosis of PAD in a diabetic foot ulcer patient is performed with the abovementioned noninvasive arterial studies and most commonly performed in a certified vascular lab. When the diagnosis of ischemia is made, or in the uncommon situation where the diagnosis is uncertain after formal vascular lab assessment, axial imaging using contrast-based computed tomography and magnetic resonance angiography may be utilized [37]. However, while these modalities are non invasive, intravenous contrast agents are often necessary to obtain adequate imaging and these may provoke allergic or nephrotoxic reactions. A further discussion on these imaging techniques will be presented in Chaps. 5 and 20 of this textbook.

Emerging Technologies to Assess Pedal Perfusion

Several technologies are currently under development for use in the assessment of pedal perfusion. Fluorescence angiography using indocyanine green is the most developed and has shown promise in providing quantitative information about regional pedal perfusion [38]. Hyperspectral imaging may become a useful technique for quantifying tissue oxy- and deoxy-hemoglobin levels. Multimodal MRI techniques may provide for the simultaneous assessment of arterial anatomy and skeletal muscle perfusion [39]. Finally, newer nuclear strategies involving PET, and SPECT combined with CT may be developed to provide for advanced assessment of

pedal perfusion [40]. Such methods are promising, yet none has been validated for use in the initial diagnosis of PAD in the diabetic patient.

Conclusion

Peripheral arterial disease is common in the patient with diabetes, and it is a particularly significant issue in the diabetic foot ulcer patient. A missed diagnosis of arterial disease can lead to protracted wound problems and healing issues. An awareness PAD and a basic understanding of the methods used to diagnose it can help to improve outcomes in this difficult patient population.

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Imaging of Infection in the Diabetic Foot

5

Mary G. Hochman and Caitlin Connolly

Abstract

Information derived from imaging studies can play an important role in the management of complicated foot problems in the diabetic patient. This chapter reviews the various modalities available for imaging of the diabetic foot—radiography, nuclear medicine studies such as bone scans, labeled leukocyte scans, bone marrow scans, and FDG PET scans, cross-sectional studies such as MRI, CT, and ultrasound, and various forms of catheter and noninvasive angiography—and highlights their relative strengths and weaknesses for the diagnosis of osteomyelitis, soft tissue infection, and neuroarthropathy. A suggested imaging algorithm for the diagnosis of osteomyelitis in the diabetic foot is presented.

Introduction

Foot infections are among the most common causes of hospitalization in the diabetic population, accounting for 20% of all diabetes related admissions. Complicated foot infections may require treatment by amputation—as many as 6–10% of all diabetic patients will undergo amputation for treatment of infection [1–3], accounting for 57% of nontraumatic lower extremity amputations [4–6]. The scope of the problem is compelling. Infections and complicated vascular diabetic foot problems result in 50,000 amputations a year in the United States [7]. The Centers for Disease Control and Prevention (CDC) estimated the annual treatment cost of amputees within this group at $1.2 billion for the year of 1997. However, this figure does not include the cost of rehabilitation, prosthetic devices, or lost income. These treatment costs are likely to grow, as the prevalence of diabetes is on the rise. A recent epidemiology study showed an increase of the overall prevalence of diabetes in the United States from 12.1 million in 2002 to 17.5 million in 2007 [8]. In 2013, the prevalence of diabetes was estimated to be 382 million people worldwide and this number was projected to rise to 592 million by 2035 [9].

Information derived from imaging studies can play an important role in management of complicated foot problems in the diabetic patient. Soft tissue abnormalities such as abscesses and cellulitis can be identified, osteomyelitis can be detected, the extent of abnormal marrow can be depicted, neuroarthropathic changes can be diagnosed and followed over time, distribution of atherosclerotic lesions can be mapped, and the effectiveness of re-vascularization procedures can be evaluated. A variety of studies are currently available for imaging the diabetic foot. In order to use these imaging studies effectively, it is important to understand the specific strengths and weaknesses of each modality, as they apply to the particular clinical problem in question. The goal of this chapter will be to review the modalities available for imaging of diabetic foot infection and to highlight their relative utilities in the context of clinical problem-solving.

Infection in the Diabetic Foot

Risk Factors

Many factors contribute to infection in the diabetic foot, including peripheral neuropathy [10] and vascular insufficiency [11]. Repetitive minor trauma to an insensitive neuropathic foot, exacerbated by abnormal biomechanics or ill-fitting shoes, causes areas of increased plantar pressure to develop callus, which, in turn, predisposes to ulcer develop-

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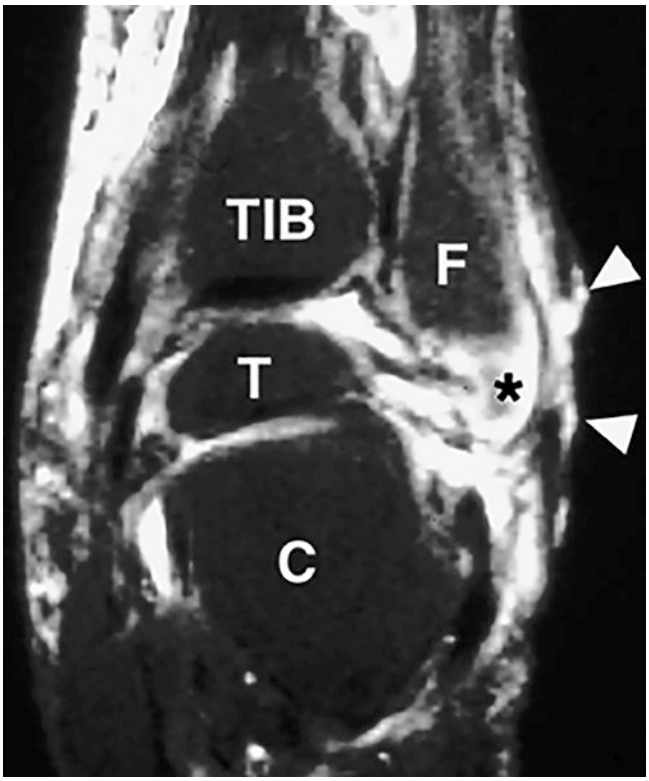

Fig. 5.1 Osteomyelitis deep to ulcer on MRI. Coronal fluid-sensitive STIR image of the left foot of a diabetic patient shows an area of marrow edema (*) at the tip of the fibula (F). Overlying this focus of abnormal marrow is an ulcer surrounded by diffuse soft tissue swelling (arrowheads). These findings represent osteomyelitis of the distal fibula. *C* calcaneus, *TIB* tibia, *T* talus

ment. Clinically occult ulcers form insidiously, deep to the callus [12, 13]. Direct extension of infected ulcers or soft tissue infection to bone leads to osteomyelitis [14] (Fig. 5.1). These infections are usually polymicrobial and involve both anaerobic and aerobic pathogens.

Soft Tissue Abnormalities

Soft tissue abnormalities associated with the diabetic foot include soft tissue edema, cellulitis, soft tissue abscess, ulcers, sinus tracts, tenosynovitis, joint effusions, and arthritis [15–17]. The importance of differentiating these conditions lies in their differing management: abscess necessitates prompt surgical drainage, septic arthritis requires surgical debridement, and cellulitis generally entails antibiotic therapy.

Soft tissue edema and swelling is a common finding in the diabetic patient. Soft tissue swelling can occur in the absence of infection, due to vascular insufficiency or peripheral neuropathy [17] (Fig. 5.2). However, soft tissue swelling can also reflect the presence of cellulitis, that is, soft tissue infection of the superficial soft tissues. Cellulitis along the dorsum of the foot usually occurs secondary to surface infections in the nails, toes or web spaces. Simple cellulitis is generally diagnosed clinically, without the need for imaging. The major indication for imaging of patients with cellulitis is suspected underlying deep infection, such as soft tissue abscess, osteomyelitis, or septic arthritis.

Osteomyelitis

Osteomyelitis of the foot occurs up to 15% of diabetic patients [16, 17]. Bone infection results from local extension of soft tissue infection (Fig. 5.1). Callus and ulcers serve as the conduits for infection to spread to deep soft tissue compartments, bones and joints. The most common sites of soft tissue infection and secondary osteomyelitis are foci of increased plantar pressure, such as the metatarsal heads and the calcaneus (Fig. 5.3). Evaluation of foot ulcers is important because more than 90% of osteomyelitis cases result from contiguous spread of infection from soft tissue to bone [7]. Newman et al. further demonstrates a clear relationship between ulcer depth and osteomyelitis: 100% of ulcers exposing bone and 82% of moderately deep ulcers were shown to have osteomyelitis on bone biopsy [1] (Fig. 5.1).

Identification of osteomyelitis in the diabetic foot can be difficult both clinically and radiographically. Ability to probe a pedal ulcer through to bone (Fig. 5.3) has been reported as a useful index of underlying osteomyelitis in a diabetic patient [18] and is commonly used to guide decisions regarding treatment. Nonetheless, clinical judgment was shown to be a poor indicator of infection. The technique of probing to bone, only 68% sensitive, may underestimate the incidence of bone involvement, according to Newman et al. [1] In the same study, 18 out of 19 of pedal ulcers did not expose bone nor display inflammation, yet contained osteomyelitis. In a recent study by Mutluoglu et al., sensitivity and specificity of the probe to bone test was 66% and 84% respectively, positive predictive value was 87%, but negative predictive value was only 62% [19]. In a study by Lavery Armstrong et al., the probe to bone test in a population of diabetic individuals with a prevalence of osteomyelitis of 12%, had a relatively low positive predictive value (0.57) [20]. A recent meta-analysis yielded a pooled sensitivity of 0.87 (95% confidence interval 0.75–0.93 and 0.83 (95% CI 0.65–0.93) [21]. Moreover, other clinical parameters such as fever and leukocytosis are unreliable in the diabetic patient. For example, in a study by Bamberger et al., only 18% of patients with clinically severe osteomyelitis were febrile [14]. Neither fever nor leukocytosis predicts the necessity for surgical exploration [22].

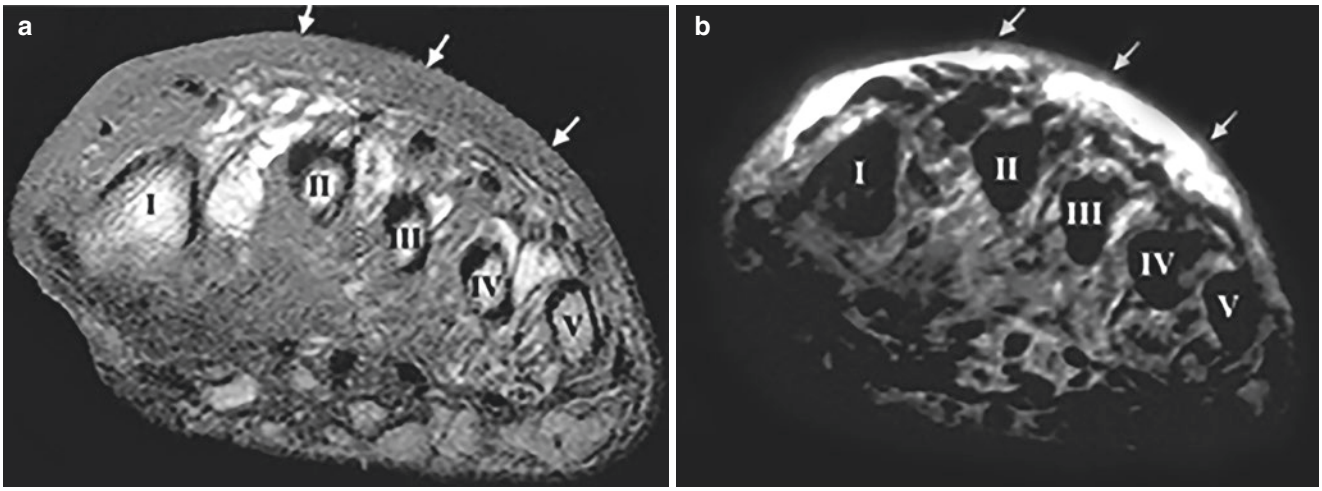

Fig. 5.2 Dorsal soft tissue swelling on MRI. (**a**) T1-weighted image and (**b**) fluid-sensitive STIR image are coronal or short axis images acquired at the level of the mid metatarsal shafts. This diabetic patient has diffuse dorsal soft tissue swelling (small arrows). The subcutaneous edema is dark or low signal on the T1-weighted image and bright or high signal on STIR. Note the presence of normal fatty marrow signal in the metatarsal bones—high signal (bright) on T1 and low signal (dark) on STIR, conclusively ruling out osteomyelitis. I–V—first to fifth metatarsals

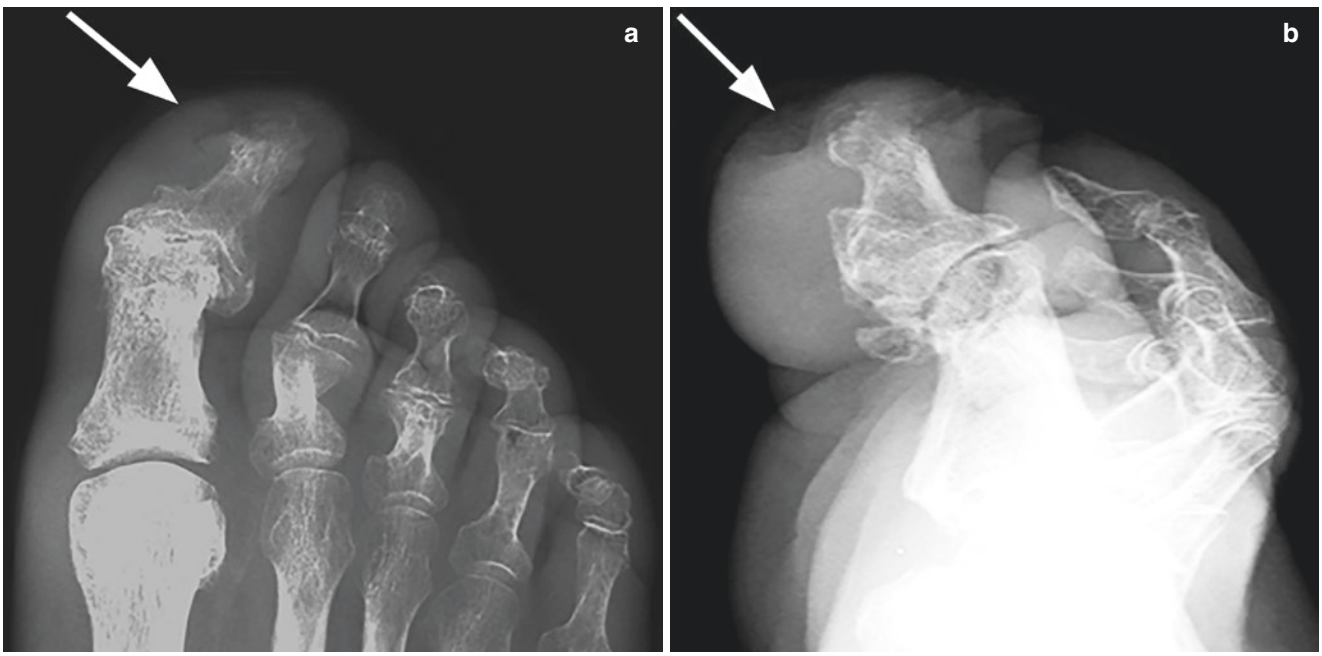

Fig. 5.3 Osteomyelitis of first distal phalanx. (**a**) AP and (**b**) lateral views of the great toe show an ulcer (arrow) overlying the distal phalanx. The cortex of the bone is indistinct and there is underlying osteopenia, representing osteomyelitis. On clinical exam, exposed bone was evident at the ulcer

Imaging Modalities

Imaging can play a role in diagnosing and distinguishing between bone and soft tissue infection, characterizing soft tissue abnormalities, identifying osteoarthopathy and other bony abnormalities, and mapping vascular disease for surgical intervention. A variety of imaging modalities can be useful in the evaluation of the diabetic foot, including radiography, scintigraphic examination, computed tomography (CT), magnetic resonance imaging (MRI), ultrasound (US), and angiography. Imaging techniques vary in their sensitivity for detection of osteomyelitis, with specificity limited in the presence of cellulitis, peripheral ischemia, and diabetic neuropathic osteoarthropathy [23, 24] (Table 5.1). In the appropriate setting, however, noninvasive imaging can aid in diagnosis and treatment planning.

Table 5.1 Compilation of sensitivity and specificity of various imaging modalities in the diagnosis of osteomyelitis

	Range of sensitivity (%)	Range of specificity (%)	Compiled sensitivity/specificity (%/%)	References
Radiography	52–93	33–92	61/72	[1, 34, 35, 101, 103, 104]
Three-phase bone scan in patients without bone complications			94/95	[31] (review of 20 published reports)
Three-phase bone scan in patients with bone complications			95/33	[31]
In-111-labeled WBC	75–100	69–100	93/80	[31, 34, 35, 36, 39]
Combined gallium and bone scan			81/69	[31]
MRI	29–100	67–95	96/87	[10, 101–104]

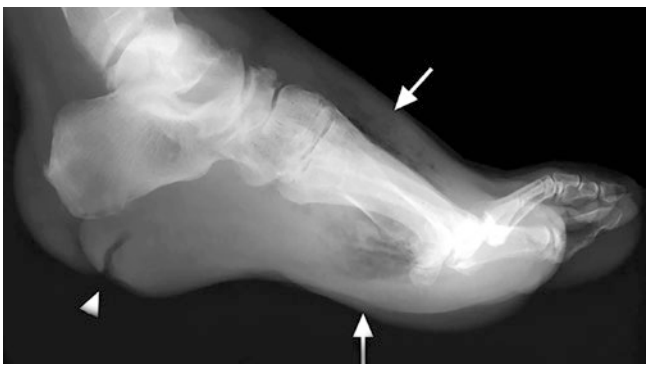

Fig. 5.4 Soft tissue air and deep ulcers on radiography. The lateral view of the right foot from a diabetic patient shows subcutaneous air (arrows) in both dorsal and plantar soft tissues surrounding the metatarsals. A deep ulcer dissects into the heel fat pad (arrowhead)

Radiographs

Radiography ("X-ray") remains the first screening examination in any patient with suspected infection and has the advantage of being inexpensive and easily obtainable. Radiographs can help to identify an unsuspected diabetic patient by demonstrating calcification in the interdigital arteries: these vessels rarely calcify in nondiabetic patients [25]. Cellulitis results in increased density and thickening of the subcutaneous fat, though nonspecific soft tissue edema can have a similar appearance. Both bone and soft tissue infection can result in blurring of usually visible fat planes. Focal fluid and soft tissue callus both demonstrate focal increased density in the subcutaneous fat. Ulcers may or may not be visible on radiographs, depending on their size and orientation (Fig. 5.4). In general, all of these soft tissue abnormalities are more clearly evident at physical exam. However, radiographs do readily depict subcutaneous emphysema associated with infection or recent surgery (Fig. 5.4). Some foreign bodies, i.e., denser materials such as metal and lead-containing glass, are radiopaque and generally are visible on radiographs. In order to detect nonmetallic foreign bodies and subtle soft tissue calcifications, radiographs acquired with "soft tissue" technique (i.e., lower kV than a routine radiograph) may be required.

Table 5.2 Radiographic findings of acute osteomyelitis

Soft tissue swelling and effacement of soft tissue fat planes
Permeative medullary radiolucency
Focal osteopenia or focal osteolytic lesion
Periosteal new bone formation
Endosteal scalloping
Cortical bone destruction

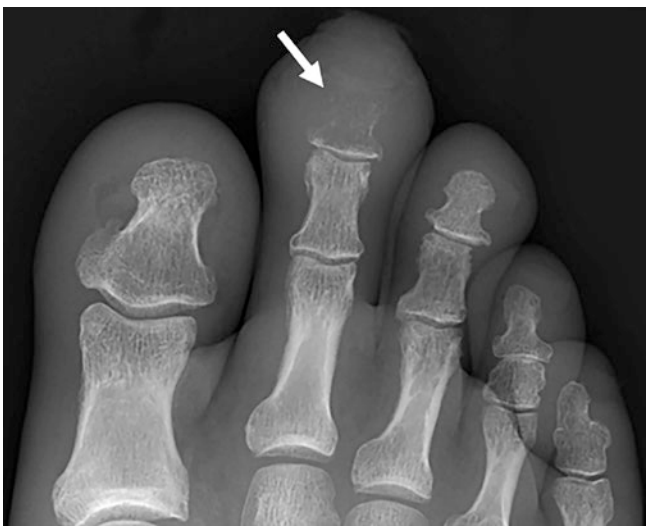

Fig. 5.5 Osteomyelitis of the second distal phalanx. Extensive destruction of cortical and medullary bone (arrow), with surrounding soft tissue swelling

Findings of osteomyelitis on radiographs include soft tissue swelling and effacement of tissue fat planes, permeative medullary radiolucency, focal osteopenia or focal osteolytic lesion, periosteal new bone formation, endosteal scalloping, and cortical bone destruction (Table 5.2, Figs. 5.3, 5.4, and 5.5). Of note, these osseous changes typically only become apparent after osteomyelitis has been present for 10–14 days and require up to 50% bone loss before becoming evident on a radiograph [26]. Comparison to prior films, when available, can help to highlight early changes. In the majority of

studies, sensitivity of radiographs ranges between 52 and 93% and specificity ranges between 33 and 92% for detection of osteomyelitis (Table 5.1). When radiographs are positive for osteomyelitis, further imaging studies are often not required for diagnosis. However, radiography is less sensitive compared with other imaging modalities and a negative X-ray examination does not exclude osteomyelitis. Moreover, radiographs are not sensitive for detection of soft tissue infection, such as septic arthritis or abscess formation. radiographs

Even when radiographs do not demonstrate findings of osteomyelitis, they nonetheless play an important role in the diagnostic workup of infection. Because they demonstrate changes of neuroarthropathy, postsurgical changes, fractures, foreign bodies, gas, foot deformities, and bony variants, radiographs can serve as roadmaps for other imaging exams. In the absence of correlative radiographs, these findings can cause unnecessary confusion on MRI or nuclear medicine exams.

Nuclear Medicine

Nuclear medicine examinations are based on administration, typically injection, of radioactive materials into a patient and measurement of resultant radioactive counts that accumulate at different sites, using a gamma camera, thereby providing a measure of activity at that site. Different types of studies are designed for specific applications based on the different materials that are labeled, for example, components of hydroxyapatite, white blood cells, sulfur colloid, and glucose. The most commonly employed nuclear medicine or scintigraphic tests for the diagnosis of diabetic foot infection are bone scans, labeled leukocyte scans, and bone marrow scans. Gallium scans are no longer commonly employed for this application. Flourine-18-flourodeoxyglucose positron-emission tomography (FDG PET) has shown utility for diagnosing musculoskeletal infection, but is not yet routinely reimbursed in the United States for this indication. Bone scans and labeled leukocyte scans are both considered highly sensitive to the presence of both soft tissue infection and osteomyelitis (Table 5.1). Traditionally, bone scans have been considered the scintigraphic exam of choice when the foot was radiographically normal, while labeled leukocyte scans were considered to provide improved specificity in cases where preexisting bone changes were present (i.e., neuroarthropathy, trauma, degenerative changes) (Table 5.1). However, the role of bone scan in the workup of osteomyelitis has shifted over time [27]. Based on current guidelines from the American College of Radiology (ACR), among nuclear medicine studies, labeled leukocyte scans are now considered the first-line imaging study of choice for the workup of suspected osteomyelitis, supplemented by a Tc-99m sulfur colloid marrow scan in those cases where background neuroarthropathic changes are present [28]. In addition, in recent years, augmentation of nuclear medicine studies by the use of three-dimensional imaging techniques such as scintigraphic SPECT (single-photon emission computed tomography) images or a hybrid technique comprised of scintigraphic SPECT images, together with conventional CT images, in the form of SPECT/CT, has also become common [29, 30].

Bone Scan

Traditionally, triple phase bone scan (TPBS) has been the test used for the workup of suspected osteomyelitis in patients with negative radiographs. It is widely available and easy to perform. A three-phase bone scan involves intravenous injection of radioactive technetium-99m methylene diphosphonate, followed by imaging with a gamma camera at three distinct time points. Images acquired every 2–5 s immediately following injection provide a radionuclide angiogram (the flow phase) and may demonstrate asymmetrically increased blood flow to the region of interest. The tissue or blood pool phase is obtained within 10 min and reveals increased extracellular fluid seen in conjunction with soft tissue inflammation. A delayed, skeletal phase is acquired 2–4 h after the injection. The skeletal phase demonstrates areas of active bone turnover, which have incorporated the radionuclide tracer, and are seen as focal "hot spots" of increased tracer activity. The tracer is taken up by bone in an amount dependent on both the degree of osteoblastic activity and the blood flow to the area. In some facilities, single-photon emission computer tomography (SPECT) spanning can be performed in conjunction with a technetium bone scan to generate tomographic, cross-sectional images of radionuclide activity that can be reformatted into different planes and can help to clarify problems created by bony overlap. Because SPECT images have greater intrinsic contrast than routine planar images, the SPECT images are also more sensitive in detecting foci of radionuclide activity.

Osteomyelitis results in increased uptake in all three phases of a bone scan, whereas simple cellulitis demonstrates increased uptake in the first two phases only (flow and tissue or blood pool phases) (Fig. 5.6). In cellulitis, there may be mild diffuse increased uptake in the bone due to inflammation, but this is distinct from the more focal, intense increased uptake seen with osteomyelitis. However, uptake in the delayed phase itself is not specific for osteomyelitis. In general, a positive delayed phase scan is seen when there is an underlying process that promotes bone remodeling, e.g., healing fracture, neuropathic osteoarthropathy, or recent bone surgery. False negatives may occur when the radiotracer fails to reach the foot because of diminished vascular flow. This is of particular concern in diabetics with atherosclerotic disease.

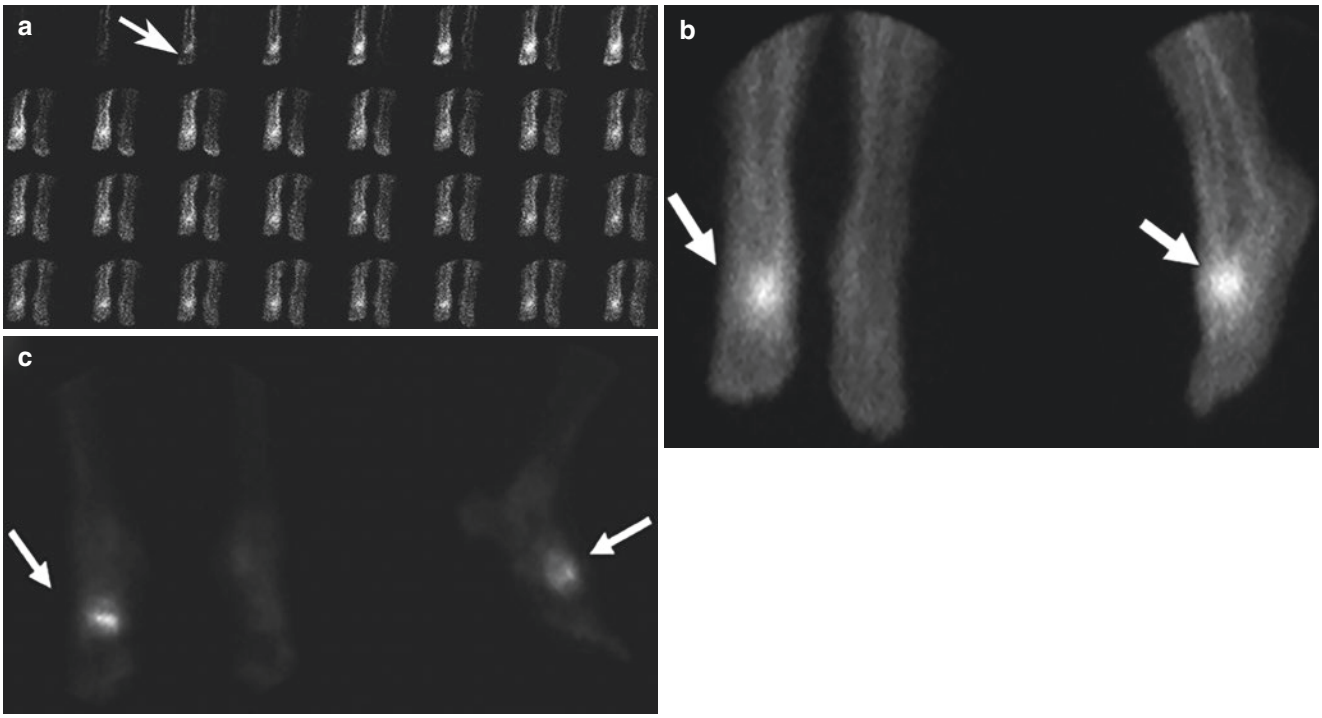

Fig. 5.6 Osteomyelitis on triple phase bone scan. (**a**) Radionuclide angiogram (flow phase) of a triple phase bone scan with successive images obtained every 2–5 s following injection, showing asymmetrically increased blood flow to the distal right lower extremity (arrow). (**b**) Blood pool phase obtained within 10 min after injection shows increased activity in the right foot (arrows), reflecting increased extracellular fluid related to soft tissue inflammation. AP view on the left and lateral view on the right. (**c**) Delayed skeletal phase acquired 2–4 h after injection shows increased activity in the bones of the midfoot. In this phase, "hot spots" reflect areas of active bone turnover (arrows) and is therefore specific for bone. Note that the signal seen in the soft tissues on the preceding blood pool phase has cleared. AP view on the left and lateral view on the right

Schauwecker's review of 20 published reports shows a compiled mean sensitivity and specificity of 94% and 95%, respectively, for detection of osteomyelitis with bone scintigraphy [31]. Unfortunately, this data applies only to patients who do not have underlying bone deformities. In the diabetic patient with complicated bone conditions such as recent fractures and neuroarthropathic changes, a common clinical presentation, the sensitivity remains at 95%, but the specificity declines to 33% [31]. Labeled leukocytes are more accurate for osteomyelitis [28, 32]. Thus, the most recent version (2012) of the American College of Radiology (ACR)-sponsored appropriateness criteria for imaging workup of osteomyelitis recommends labeled leukocyte scans as the first-line nuclear medicine imaging study and describes a three-phase bone scan as "usually inappropriate" in the imaging workup of osteomyelitis, though they do note that a negative bone scan has a high negative predictive value and excludes infection with a high degree of certainty [28]. Nonetheless, three-phase bone scans may still be obtained in some clinical practices, especially when radiographic findings of background bone complications, such as fractures and changes of neuro-osteoarthropathic changes, are absent [28].

Labeled Leukocyte (White Blood Cell) Scan and Bone Marrow Scan

Labeled leukocyte scans, also known as labeled white blood cell (WBC) scans, are the preferred scintigraphic technique for imaging when there is background bone pathology on radiographs. This is because WBCs accumulate at sites of infection, but, unlike bone scans, they theoretically do not accumulate at sites of increased bone turnover, such as fractures and neuropathic osteoarthropathy. White blood cell scans are performed by extracting a patient's blood, fractionating the leukocytes from blood, incubating the white blood cells with the either indium 111-oxine or technetium-99m-hexamethylpropylene amine oxime (Tc99m-HMPAO) in order to label them, and then re-injecting the labeled white blood cells into the same patient. Imaging is performed 16–24 h later for indium-labeled leukocytes and 3-4 h later for Tc99m-HMPAO, using a standard gamma camera. As noted above, labeled white blood cells theoretically only accumulate at sites of infection and not at sites of increased osteoblastic activity and therefore should be extremely useful in the diagnosis of osteomyelitis complicated by underlying bone changes (Fig. 5.7). The technique is most useful for inflammatory processes that are mediated by neutrophils,

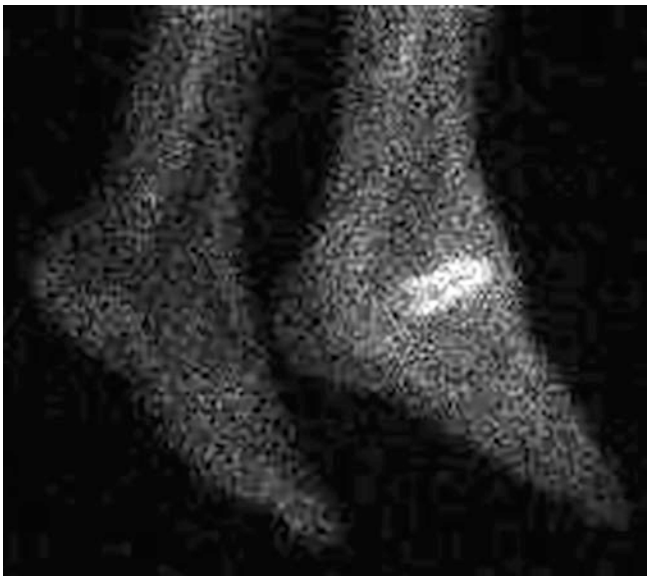

Fig. 5.7 Osteomyelitis on indium-labeled leukocyte scan. Increased indium accumulation about the ankle represents a focus of osteomyelitis in a patient with swelling and fever. Staph aureus grew from the marrow aspirate

such as bacterial infections, since the majority of leukocytes labeled are neutrophils [33]. In addition, a total white count of at least 2000/μL is needed to obtain satisfactory results [33].

Indium-labeled leukocyte scan offers the best sensitivity and specificity for detection of osteomyelitis, compared to triple phase bone scans and gallium scans (Table 5.1). A compilation of seven studies yielded a sensitivity of 93% and specificity of 80% [1, 34–39]. In addition, Newman suggested that indium-labeled leukocyte imaging could be used to monitor response to therapy, with images reverting to normal 2–8 weeks after commencement of antibiotic therapy [1].

Because of their potential advantages and reported high sensitivity and specificity, indium-labeled leukocyte scans are considered the radionuclide test of choice for evaluation of suspected osteomyelitis in the diabetic foot [32]. In the most recent American College of Radiology (ACR)-sponsored appropriateness criteria guidelines for the imaging detection of osteomyelitis in patients with diabetes mellitus and without neuropathic arthropathy [28], a labeled leukocyte (white blood cell) scan was deemed "may be appropriate in certain circumstances, such as if an MRI is contraindicated or unavailable" the highest rating given to a nuclear medicine study. The sensitivity and specificity of planar Indium-111 WBC scan ranges from 72 to 100% and from 67 to 100%, respectively [32]. However, early data had shown false-positive uptake of indium-labeled leukocytes in as many as 31% of noninfected neuropathic joints [40]. These false-positive examinations stemmed from the inability to determine whether labeled leukocytes located outside the typical marrow distribution represents infection or merely an atypical site of hematopoietic activity [41]. Atypical patterns of marrow distribution may accompany fractures, orthopedic hardware, infarctions, systemic diseases, neuropathic joints, and tumors, and make it difficult to distinguish WBC activity due to osteomyelitis. At sites where confounding bone marrow may be present, it is very helpful to compare the leukocyte scan to a second separate nuclear medicine study—a scintigraphic scan of bone marrow obtained with technetium-99m-sulfur colloid. This strategy is based on the fact that both WBCs and sulfur colloid accumulate in marrow, independent of its location, but only WBCs—not sulfur colloid—accumulate in bone infection. Thus, the sulfur colloid bone marrow scan maps out the distribution of normal bone marrow, even when it is an atypical distribution [42]. A combination of leukocyte and bone marrow scans is positive for osteomyelitis when it demonstrates radionuclide uptake that is greater, in either intensity or distribution, on the leukocyte scan than on the bone marrow scan (i.e., "incongruent" scans) (Fig. 5.8). For this reason, in the setting of neuroarthropathy or other confounding bone changes, interpreting a labeled leukocyte scan together with the results of a bone marrow scan in the setting of improves accuracy and specificity for detection of osteomyelitis [42, 43]. The combined study has been reported to be 88–98% accurate [42]. Reflecting this, the ACR appropriateness criteria therefore state that, in the setting of neuropathic arthropathy and suspected osteomyelitis, a labeled leukocyte scan obtained *in conjunction with* a bone marrow scan "may be appropriate in select clinical circumstances" (highest rating given to a nuclear medicine study) [28]. In practice, when white blood cell scans are labeled with indium-111 and bone marrow scans are labeled with technetium-99m, both scans can be obtained simultaneously, during one sitting, because the two different radionuclides have different energies and they can be distinguished by the gamma camera, by using different energy "window settings" for collecting the radioactivity counts. Limitations of combined WBC and sulfur colloid scans include: absence of WBC activity in the area of interest, in which case marrow imaging is not productive; sulfur colloid images become osteopenic about 1 week after onset of infection; if Tc99m-sulfur colloid is not properly prepared or has been prepared more than 2 h prior to imaging, image quality will be degraded, limiting accurate assessment; labeled WBCs may accumulate in lymph nodes, though the lymph node activity can typically be distinguished by its characteristic morphology and distribution along the lymph node chain [42]. It is also important to recognize that the distribution of marrow changes with age.

Detection of osteomyelitis is rarely a problem in the forefoot, where the osseous structures are equidistant from both dorsal and plantar skin surfaces, but may be compromised in

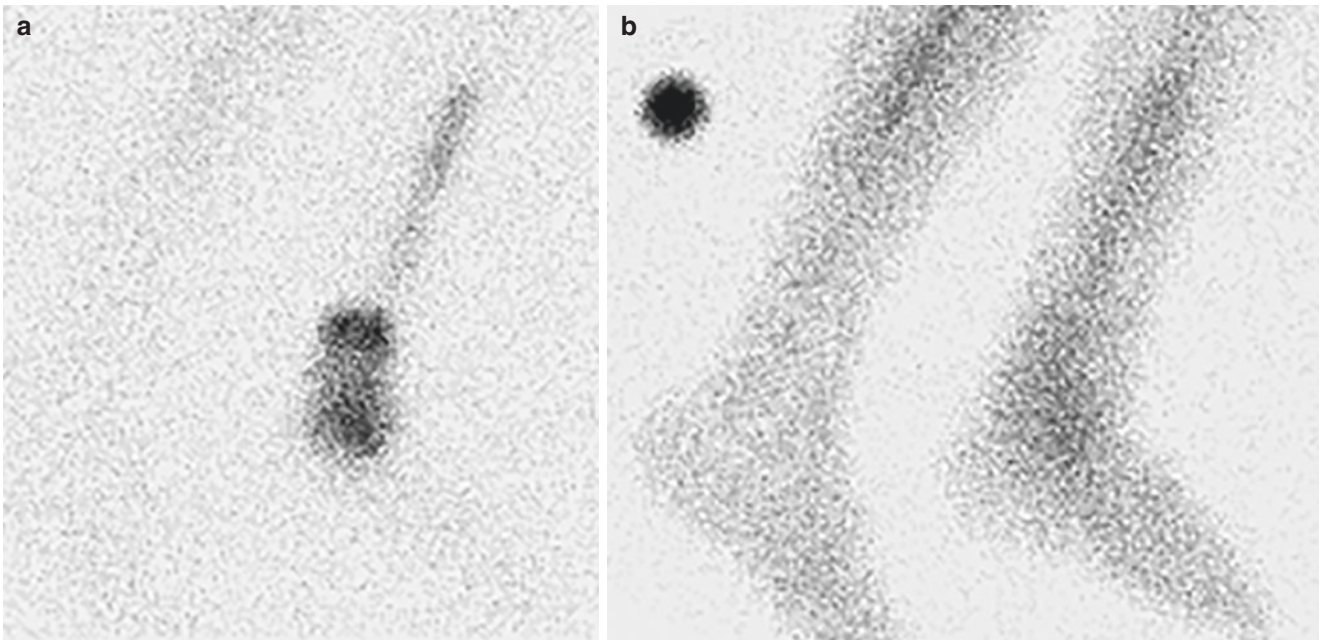

Fig. 5.8 Indium-111-labeled leukocyte scan and technetium-99m-labeled sulfur colloid scan demonstrating incongruence indicative of osteomyelitis. (**a**) Indium-111-labeled leukocyte scan shows increased activity in the tibia and ankle in a diabetic patient with a nonhealing ulcer over the tibia. (**b**) Technetium-99m-labeled sulfur colloid scan shows increased activity in a similar distribution, but significantly less intense than that seen on the leukocyte scan, demonstrating an "incongruent scan" indicative of osteomyelitis

the mid- and hindfoot due to anatomic complexity in these areas [33]. Interpreting the labeled leukocyte scan in conjunction with the anatomic localizing information available from a simultaneously acquired SPECT/CT can help to improve accuracy in diagnosing osteomyelitis [29, 30, 32]. SPECT/CT refers to fusion of scintigraphic and morphologic images into a hybrid imaging study comprised of a nuclear medicine single-photon emission computed tomography scan together with a conventional radiographic CT scan depicting 3D anatomy, which provides anatomic landmarks for the areas of increased nuclear medicine uptake, and which has contributed to improved diagnostic accuracy over SPECT alone in many scintigraphic procedures [30] (Fig. 5.9). In this way, for example, SPECT/CT can aid in decreasing false positives due to mis-localized uptake in the soft tissues [32]. SPECT/CT has also demonstrated utility in evaluating for response to treatment of osteomyelitis [44, 45] and potentially for categorizing severity and likelihood of response to treatment [46]. If SPECT/CT is unavailable, a contemporaneous bone scan can help to improve accuracy of the labeled leukocyte scan [31].

Other disadvantages associated with indium-labeled leukocyte scans include the complexity of the labeling process, which can result in false-negative examinations if the procedure for labeling the leukocyte is inadequate [29]; high costs; limited availability of the test; and the risks inherent in handling of blood products [29]. Because of the difficulties inherent in in vitro labeling of leukocytes, several techniques for in vivo-labeled leukocyte imaging have been developed. However, these techniques are not at present in widespread use [33].

Gallium Scan

Gallium is not frequently used in workup of diabetic pedal osteomyelitis. In the past, it had been described as a useful alternative for assessment of pedal infection when there were abnormal radiographic findings on a foot radiograph and a labeled leukocyte scan or MRI was not available. However, in recent years, indium-labeled leukocyte scans, often in conjunction with bone marrow scans, have in large part supplanted the use of gallium-67 scans in this setting. At present, gallium studies are not included in the American College of Radiology's most recent guidelines for the imaging workup of osteomyelitis in the diabetic foot [28].

FDG PET Scan

Flourine-18-labeled fluorodeoxyglucose (FDG) imaging using positron emission tomography (PET) has become an important technique for oncologic imaging and is in common clinical use for detection, staging, and monitoring response to therapy in lung cancer, breast cancer, lymphoma, and melanoma, among others [47]. However, FDG PET scans often also show increased activity in areas of inflammation or infection and the use of PET for these non-neoplastic applications is now being actively investigated [47]. At this juncture, however, FDG PET exams are not

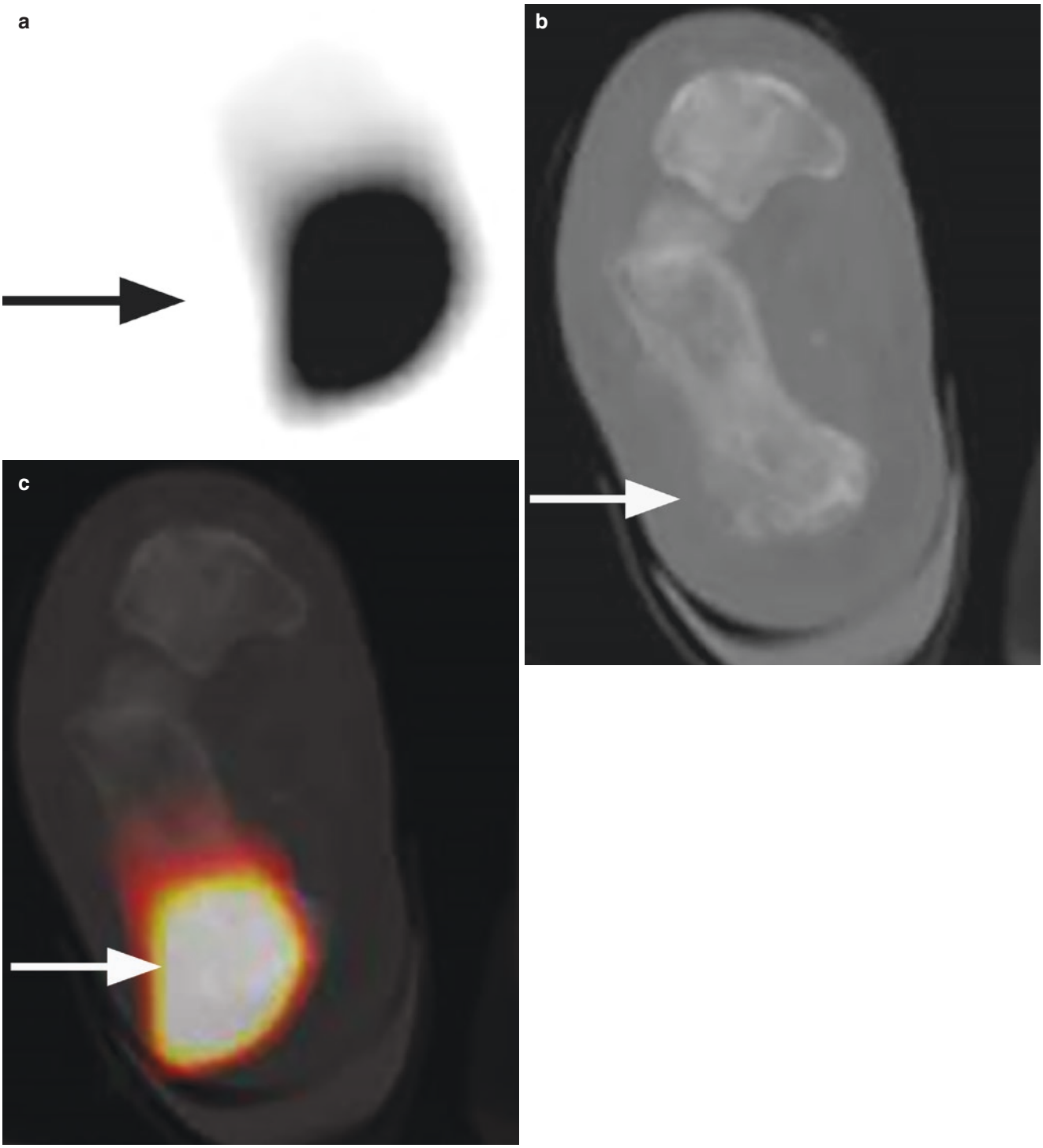

Fig. 5.9 Osteomyelitis of the calcaneus on indium-labeled leukocyte scan. (**a**) Axial image of the hindfoot obtained using SPECT single-photon emission computed tomographic technique shows increased leukocyte uptake in the hindfoot (arrow). (**b**) Axial CT image shows cortical irregularity of the lateral calcaneus, reflecting bone erosion due to osteomyelitis (arrow). (**c**) The radionuclide WBC scan image is superimposed onto the CT image, resulting in a fused SPECT/CT image that clearly localizes the leukocyte uptake to the calcaneus (arrow)

routinely reimbursed for applications related to infection in the United States.

FDG is a radiolabeled glucose analogue that is taken up by cells in proportion to their metabolic rate and number of glucose transporter proteins. Increased FDG uptake is seen in

inflammation, due to increased expression of glucose transporters and increased affinity for the glucose analogue by activated inflammatory cells. The fluorine-18 (^{18}F) radionuclide is produced in a particle accelerator known as a cyclotron and has a relatively short radioactive half-life. After intravenous injection of flourine-18 FDG, a patient is imaged 30–60 min later using a PET scanner. A routine exam includes images from the level of the skull base through the mid-thigh, though examinations spanning the skull to the feet can be performed. Areas of increased activity on the images reflect sites of increased glucose metabolism and may be described in terms of standardized uptake value (SUV). Many of the scanners currently being installed are PET-CT scanners, which incorporate both a PET scanner and a conventional CT scanner, In a PET-CT system, PET and conventional CT images are both obtained during the same examination and can be fused together into hybrid images, to aid in localization of areas of increased activity. This improved localization capability can be used, for example, to help distinguish between osteomyelitis and soft tissue infection [48].

FDG-PET has shown some promising results for imaging of infection, but remains an investigational technique. In general, sensitivity for infection tends to be relatively high and negative predictive value is very high, but false positives can occur because any area of increased metabolic activity—not just infection—will show increased radionuclide activity. Recent surgery can also result in false-positive increased activity [47]. Chacko et al. examined 167 PET scans in 175 anatomic sites and found an accuracy of 91.2% for chronic osteomyelitis [49]. Meller et al. prospectively compared FDG PET and labeled leukocytes and concluded that FDG was superior for the diagnosis of chronic osteomyelitis [50]. PET has also shown utility in evaluation of chronic osteomyelitis and infected prostheses [51]. In a meta-analysis by Termaat et al., FDG PET shows a pooled sensitivity of 96% and a specificity of 91% for the diagnosis of chronic osteomyelitis [52]. In a limited number of cases, correlative decreases in FDG uptake and inflammatory activity have been reported following antibiotic treatment [53], suggesting a potential role in tracking response to therapy, analogous to its current use in tumor treatment [47, 54]. A series of novel PET tracers are currently being evaluated for imaging of infection and inflammation [51]. Overall, FDG-PET has shown good sensitivity for imaging of osteomyelitis [55], but is not yet reimbursed for this indication in the United States.

Specific data on the use of FDG PET for assessment of infection in the diabetic foot remains relatively limited. Keider et al. examined 18 sites of infection in 14 patients and demonstrated that FDG PET could help to precisely localize infection and could distinguish between bone and soft tissue infection in the diabetic foot [48]. In contrast, in a study by Schwegler et al. that included seven diabetic patients with chronic foot ulcers and biopsy-proven osteomyelitis, FDG was positive in only two cases, while MRI was positive in six [56]. In a meta-analysis by Treglia et al., FDG PET showed a pooled sensitivity of 74% and specificity of 91% for detection of osteomyelitis in the diabetic foot [57].

Compared with WBC scans, FDG PET offers shorter exam times and obviates the need for drawing WBCs from the patient for labeling. PET is less susceptible than WBC scans to false negatives resulting from decreased perfusion at the infection site. While PET and WBC scans are thought to be comparable in sensitivity in the peripheral skeleton (where there is usually a paucity of hematopoietic marrow to cause spurious WBC activity), PET is considered more effective than WBC scans for detection of central foci of infection/inflammation, because of physiologic uptake of WBCs by bone marrow in the axial skeleton [47]. A potential concern related to the use of PET in diabetic patients relates to the effect of chronic hyperglycemia on FDG uptake in metabolically active lesions [58]. However, a recent study suggested that mild to moderately elevated serum glucose levels do not adversely affect the accuracy of 18FDG PET in detection of pedal osteomyelitis in diabetic patients [59].

Newer Radiopharmaceuticals

A number of new radiopharmaceuticals that may have application in the diagnosis of diabetic foot infection are being investigated, but have not entered routine clinical practice. These include radiolabeled antigranulocyte antibodies, immunoglobulins, antibiotics, and radiotracers specifically taken up by bacteria [60].

Computed Tomography (CT)

CT scans can show findings of osteomyelitis earlier than radiographs, but are not considered a front-line examination for the diagnosis of osteomyelitis, because they are less sensitive than MRI for soft tissue and osseous infection and also, because, unlike MRI, they expose the patient to ionizing radiation.

Computed tomography (CT) scans use ionizing radiation to generate cross-sectional scans of the body. Tissues are displayed on a gray scale that reflects their relative X-ray attenuation, a quantity that is expressed in Hounsfield Units (HU). For example, Hounsfield units typically measure −1000 for air, 0 for water, ~40 for soft tissue, and ≥400 for bone. Most CT scans are now performed on multidetector scanners, which allow acquisition of thinner cross-sectional images and faster imaging times. When thin-section "volumetric" scans are acquired with a multidetector scanner, image sets acquired in one plane can be reformatted computationally into any desired imaging plane, after they have been acquired, e.g., images acquired axially can be reformatted into coronal or sagittal images. Image data can be post-processed with differ-

ent algorithms to highlight either bones or soft tissues. Independent of that post-processing, images can also be displayed using "bone" or "soft" tissue windows. Image data can also be post-processed to highlight anatomy in different ways, such as Maximum Intensity Projection (MIP images) to produce a CT angiogram or volume rendering (VR) to create a 3D display of various tissues.

CT scans are often performed using intravenous iodinated contrast, in order to highlight different tissues, demonstrate characteristic enhancement patterns of certain structures, outline cysts and fluid collections and distinguish them from solid masses, and depict vascular anatomy. In most cases, CT contrast administration is uneventful. However, some patients experience reactions after IV administration of iodinated contrast, with fatal anaphylactoid reactions in approximately 1 in 40,000 patients [61]. The risk of reaction is significantly reduced with low osmolar nonionic contrast, now in routine use at many institutions [62]. Use of nonionic contrast also decreases the incidence of nausea, vomiting, hemodynamic instability, and discomfort or pain associated with contrast administration, effects that are relate to the osmolality of the contrast [62, 63]. In patients with a history of contrast allergy, the 2016 version of the American College of Radiology Manual on Contrast Media (version 10.2) proposes a premedication regimen that includes oral prednisone or oral methylprednisolone, together with diphenhydramine, to reduce the frequency and/or severity of the contrast reaction, prior to elective contrast administration [64]. An optional change to a different low-osmolality contrast agent from one that has been known to cause a past allergic reaction may also be considered [64]. Patients with elevated creatinine (>1.5 mg/dL) and diabetes (especially insulin dependent diabetes) are at increased risk for contrast-induced renal failure due to acute tubular necrosis [65]. Contrast-induced nephropathy occurs with both ionic and nonionic contrast, although less frequently with nonionic forms. The overall incidence of contrast-induced renal failure is low (1–2% in patients with normal renal function) [62] and the effect is usually brief and self-limited. However, the rate is significantly higher in patients with renal failure (10% in patients with serum creatinine 1.3–1.9 mg/dL and up to 65% with levels >2 mg/dL). [62]. Moreover, contrast-induced renal insufficiency in a patient on the oral hyperglycemic agent dimethylbiguanide (Metformin) can result in fatal lactic acidosis, leading to the recommendation that Metformin should be temporarily withheld prior to and following contrast administration [62]. Intravenous hydration is used as a preventive measure in this setting; the use of diuretics may be deleterious [66]. The 2016 ACR Contrast Manual on Contrast Media distinguishes between two categories of patients on Metformin: (1) Category I, i.e., those patients with no evidence of AKI and with an eGFR ≥30 mL/min/1.73m^2, who do not require discontinuation of metformin prior to or following the administration of iodinated IC contrast; and (2) Category II, those patients who have known acute kidney injury, severe chronic kidney disease, or are planned to undergo an arterial catheter procedure that may result in emboli to the renal arteries (Category II), in whom dimethylbiguanide (Metformin) should be temporarily discontinued [64]. A more complete discussion of topics related to contrast allergy and prophylaxis and patients on dimethylbiguanide (Metformin) is available in the American College of Radiology's 2016 ACR Manual on Contrast Media [64].

Advantages of CT include high spatial resolution of CT images, superb depiction of bony detail and small calcifications, and the ability to image large areas of anatomy in a single, rapid scan. Disadvantages of CT include exposure to ionizing radiation and risks associated with contrast administration. Of note, the radiation dose from scanning extremities is significantly less than that associated with scans through the torso. Orthopedic hardware can cause "beam hardening" artifact that obscures surrounding anatomy, but, with newer generations of scanners as well as new techniques for reduction of metal artifact, the effects are less pronounced than they have been in the past [67, 68]. Nonetheless, stents, dense prostheses, and large metallic constructs can pose problems for diagnostic imaging.

During early stages of acute osteomyelitis, changes may be difficult to detect on radiography, but can frequently be documented on CT. CT is superior to radiography in detection of cortical destruction (Fig. 5.10), periostitis, and soft tissue or intraosseous gas [69, 70]. CT can also demonstrate increased density of intraosseous medullary fat and blurring of soft tissue fat planes due to the presence of pus and edema [71, 72]. CT is extremely effective in demonstrating a bony sequestrum when present in chronic osteomyelitis (a focus of necrotic bone insulated from viable bone by granulation tissue). The sequestrum appears as a dense bone spicule situated within the medullary cavity and surrounded by soft tissue density [7, 73]. CT scan is useful for detection of radiographically occult foreign bodies, even those that are not traditionally considered radiopaque (e.g., wood). While CT scans performed with intravenous iodinated contrast material can demonstrate soft tissue abscesses and necrotic tissue as areas of non-enhancement, MRI and ultrasound, imaging modalities that possess superior intrinsic soft tissue contrast resolution, are better suited to imaging of abscess collections and, when necessary, can be performed in the absence of intravenous contrast. Thus, use of CT for detection of soft tissue abscess should be weighed against the risk of contrast-induced complications. Overall, the data on sensitivity or specificity of CT for diagnosis of diabetic pedal osteomyelitis is scant. In light of concerns regarding risks of ionizing radiation, allergic reaction to contrast, and, in particular, contrast-induced nephropathy, there appears to be little enthusiasm for using CT as a routine diagnostic test for

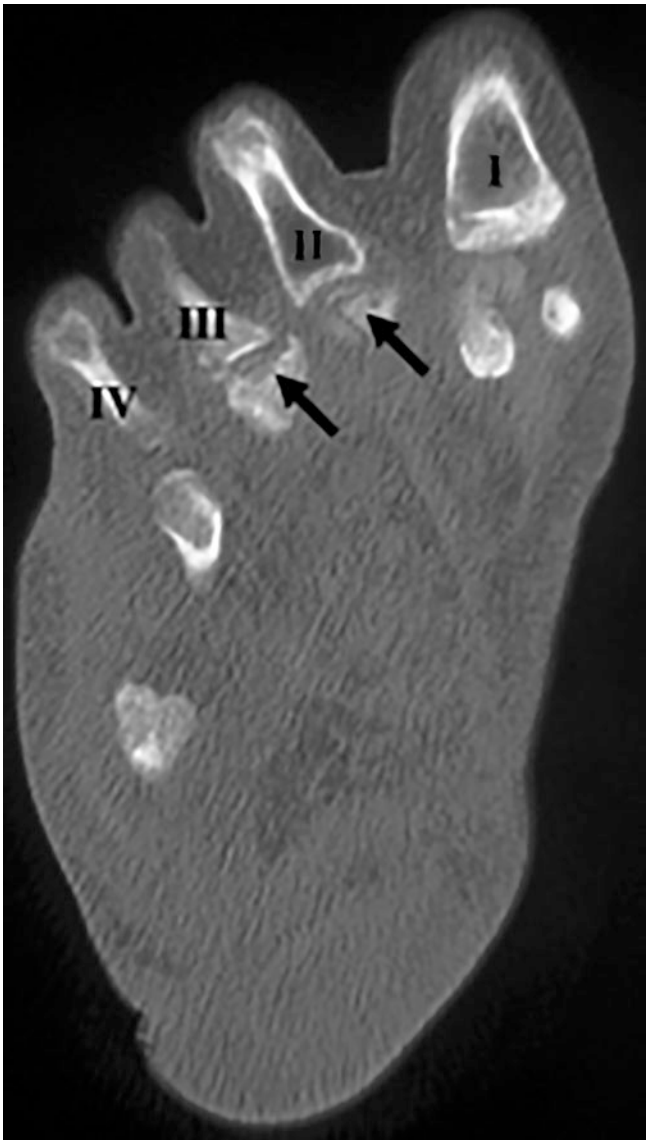

Fig. 5.10 Metatarsal Osteonecrosis on CT. The second and third metatarsal heads are flattened. The radiolucencies beneath the deformed metatarsal heads represent subchondral fractures (arrows). CT exquisitely demonstrates these cortical abnormalities

osteomyelitis. The 2012 ACR guidelines indicate that CT may be appropriate in evaluation of a patient with neuropathic arthropathy and soft tissue swelling, but no ulcer, because CT can identify more subtle, radiographically occult changes of neuroarthropathy and may be particularly useful when MRI is contraindicated [28].

Ultrasound

Grayscale ultrasound has very limited application in imaging of bone and bone infection, because of the acoustic shadowing caused by cortical bone, though ultrasound has been used to image soft tissue infection and subperiosteal abscesses and can be used to guide aspiration of soft tissue infection. (Duplex Doppler ultrasound imaging of vasculature in the diabetic foot is discussed separately below.)

Ultrasound images are produced using an ultrasound transducer to transmit and receive ultrasonic waves of given frequencies, by holding the transducer against a patient's skin [74]. The amplitude of the sound that is reflected back (rather than transmitted forward) is translated into a gray-scale image of the underlying anatomy. Areas of interest are described based on their resultant echogenicity. Areas that transmit ultrasound waves with negligible reflectance, such as simple fluid, appear uniformly dark and are termed anechoic; areas that are highly reflective of sound waves, such as cortical bone, appear bright and are termed hyperechoic. Different tissues, such as muscles, tendons, and nerves, when normal, have characteristic reflectance patterns. Diagnostic ultrasonography of the foot is performed using a high-frequency transducer, often in conjunction with a stand-off pad or its equivalent.

Ultrasound has many advantages for imaging the diabetic patient. Ultrasound examinations do not involve ionizing radiation, entail minimal patient discomfort, and can often be performed in small children without the use of sedation. Ultrasound can be performed in patients who might have contraindications to MRI and can often yield a diagnostic examination in cases where orthopedic hardware might preclude successful imaging by MRI or CT. Ultrasound equipment is relatively low cost, easily transportable, and is more widely available than MRI in many countries. Unlike many other imaging modalities, ultrasound readily provides real-time imaging and therefore can be used to assess motion and to guide aspirations, biopsies, and therapeutic injections. The major—and important—disadvantage of ultrasound is that it requires a high level of operator and interpreter expertise.

Ultrasound is well suited for evaluation of superficial soft tissues and for guiding aspiration and drainage of intra- or extra-articular fluid collections. Abscesses are seen as hypoechoic collections with increased through-transmission (that is, the tissue deep to the abscess appears more echogenic than expected, because the sound waves are attenuated to a lesser degree by the fluid in the abscess than by the soft tissue surrounding the abscess) (Fig. 5.11). However, an abscess may be difficult to identify on ultrasound when its contents become proteinaceous, because it can then become isoechoic to the surrounding tissues and may fail to demonstrate enhanced signal in the tissues deep to the abscess. Similarly, joint effusions are often visible as hypoechoic on ultrasound, but may be less evident when their contents are complex. Even when sonography demonstrates a fluid collection, the presence or absence of infection within the fluid cannot be established by imaging. Thus, ultrasound is often employed for guiding aspiration of the suspect fluid collection.

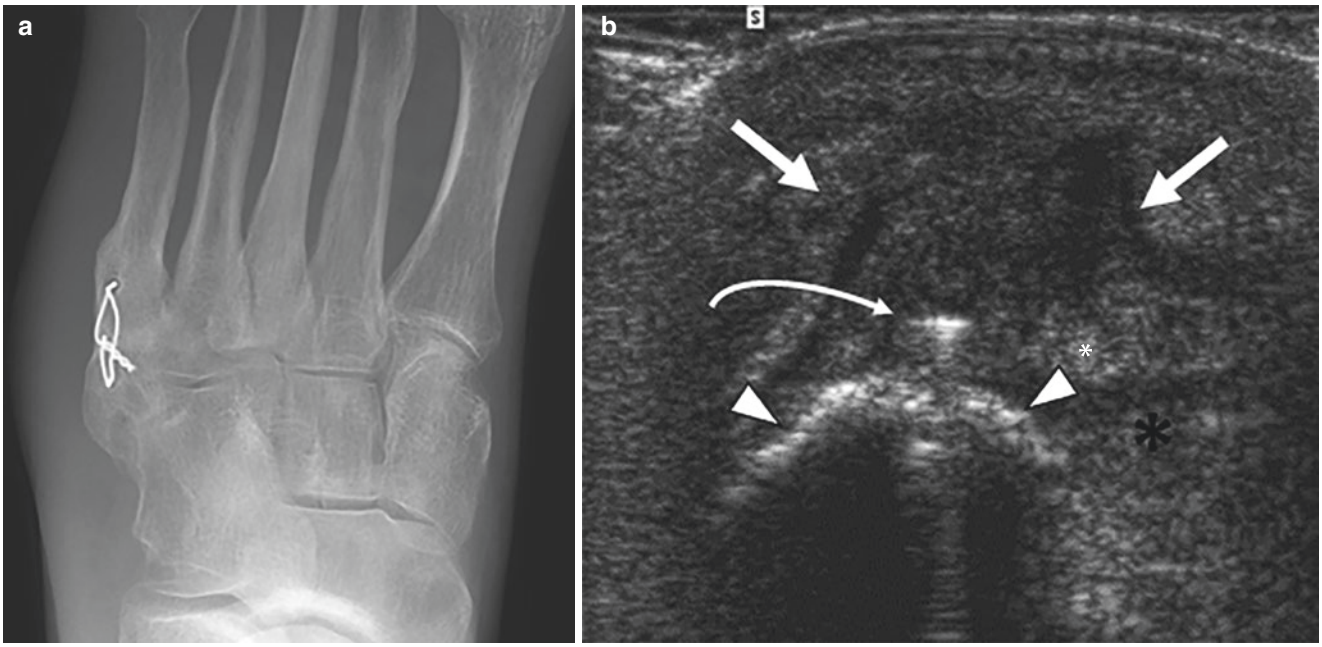

Fig. 5.11 Soft tissue abscess on ultrasound. (**a**) AP radiograph of the foot shows soft tissue swelling adjacent to the fifth metatarsal (arrow), but does not distinguish between generalized soft tissue swelling and detection of a focal abscess. (**b**) Grayscale ultrasound image obtained in cross-section to the base of the fifth metatarsal shows a complex fluid collection in the overlying soft tissues (arrows), consistent with an abscess. Simple fluid appears anechoic (dark), but more complex components are similar in echogenicity to—and harder to distinguish from—surrounding tissues. The bright, hyperechoic curvilinear line is the cortex of the bone (arrowheads). The dark, anechoic area below the cortex is caused by acoustic shadowing from the cortex and (routinely) precludes ultrasound evaluation of the medullary cavity. The small bright area immediately above the cortex (curved arrow) represents an orthopedic wire. The bright, hyperechoic area next to the bone (asterisk) represents enhanced-through transmission, a sign that the tissue above it has fluid content

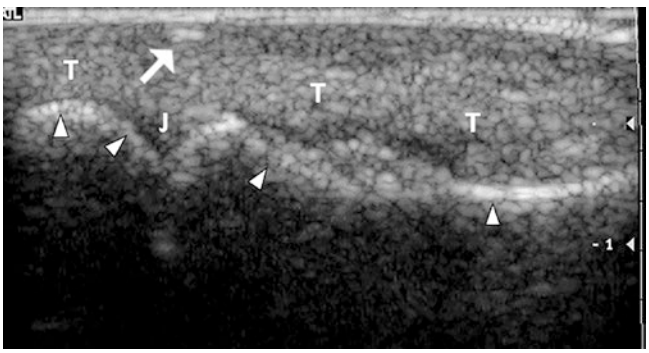

Fig. 5.12 Foreign body on ultrasound. Ultrasound image along longitudinal axis of a digit shows a small hyperechoic line (arrow) representing a small 12 mm foreign body. Thicker hyperechoic lines (arrowheads) represent the bony cortex, which obscures the underlying medullary cavity. *T* tendon, *J* joint space

Ultrasound is not very useful for direct evaluation of osteomyelitis, particularly early osteomyelitis, because cortical bone causes acoustic shadowing that obscures the underlying bone [75] (Fig. 5.12). However, in children, the use of ultrasound to demonstrate subperiosteal abscesses has been described [76, 77]. Subperiosteal abscess is a feature of osteomyelitis in children, but not adults, because, in children, the periosteum is more loosely adherent to the bone and, therefore, more easily displaced by pus. Subperiosteal abscess appears as an anechoic or moderately echoic zone >2 mm thick, adjacent to the bone and can be detected prior to changes on plain radiographs [78, 79]. Care must be taken not to mistake soft tissue abscess or soft tissue inflammatory changes adjacent to bone for subperiosteal abscess [80]. Power Doppler sonography can be used to demonstrate hyperemia surrounding a subperiosteal abscess, though it may not be positive in the early days of abscess formation [81]. Other signs associated with osteomyelitis that may be apparent at ultrasound include: fistulous communication between a subperiosteal abscess and the skin surface, swelling and edema in muscles immediately overlying the infected bone, and, in advanced cases, frank discontinuity of cortex [75, 82, 83]. Ultrasound can be very useful for detection of foreign bodies [84] (Fig. 5.12). Using ultrasound, an in vivo study of 50 patients with suspected nonradiopaque foreign bodies yielded a sensitivity of 95% and specificity of 89% for foreign body detection [85].

Because it can readily demonstrate musculoskeletal soft tissue structures and allows for accurate measurement, ultrasound has been used in a number of studies to identify correlates for degradation in biomechanical function in the diabetic foot. For example, D'Ambrogi et al. measured the thickness of the Achilles tendon and plantar fascia in 61

diabetic patients (27 without neuropathy; 34 without) and 21 healthy volunteers and found significant thickening of the plantar fascia and Achilles tendon in the diabetic patients [86]. The abnormalities were more pronounced in neuropathic patients. Hsu and Wang et al. used ultrasound to compare the heel-pad mechanical properties in Type II diabetes patients with and without forefoot ulceration against healthy controls and found higher energy dissipation ratios when exposed to a load that simulated peak standing in-shoe plantar pressures within the heel pad of patients with Type II diabetes. They speculated that this could increase risk for developing foot ulceration [87]. Naemi et al. used real-time ultrasound elastography to measure the thickness and stiffness of the heel pad in 39 patients, 10 of who had ulcerations at a site other than the heel or submetatarsal foot pad [88]. In this preliminary assessment, they found that the group with foot ulceration had a significantly lower relative stiffness of the heel pad. This latter study employed a relatively recently developed ultrasound-based technique, known as US elastography (EUS), that allows for assessment of mechanical properties of tissues. This technique is still in relatively early stages and several alternative techniques for measurement of tissue stiffness using ultrasound exist. Strain or compression ultrasound elastography involves application of a compressive force to the tissue, with resultant axial displacement of tissue (strain). Strain is calculated by comparing the data obtained before and after compression. Assuming the applied stress is uniform, the elastic modulus of the tissue is inversely proportional to the measured strain (based on Hooke's law for the calculation of Young's elastic modulus, which is a measure of the stiffness of a solid material). The relative strain of one tissue area to another is compared and displayed as a color map overlaying a grayscale anatomic image. In contrast, shear wave EUS is based on the fact that shear waves are generated within tissue by conventional ultrasound waves. These shear waves propagate perpendicular to the axial displacement caused by the ultrasound wave and the shear waves experience rapid attenuation. The velocity of these shear waves can be measured and used to calculate tissue stiffness. Shear wave EUS yields both color maps of stiffness overlaying the grayscale anatomic image and, in theory, objective quantitative maps of elasticity in (kPA) and shear wave velocity (cm/s).

Magnetic Resonance Imaging (MRI)

Technique

MRI is a primary modality for assessment of bone and soft tissue infection in the diabetic foot. Because it provides high intrinsic soft tissue contrast, MRI exquisitely depicts the full spectrum of soft tissues and can demonstrate radiographically-occult bone marrow edema, without the use of intravenous contrast. Advantages of MRI over scintigraphy are precise anatomic definition and improved lesion characterization, lack of ionizing radiation, and shorter overall exam times. Because of its high sensitivity for abnormal bone and soft tissue edema and high negative predictive value, MRI can readily detect and delineate an infection's the anatomic location and extent of an infection and can exclude infection when it is absent, making it a useful aid for surgical planning [10] (Table 5.3). Because of high sensitivity to marrow and soft tissue edema on MRI, however, it can sometimes be difficult to distinguish osteomyelitis and soft tissue infection from other causes of edema, such as fracture, early osteonecrosis, and reactive edema around an infection site. Postoperative changes can also cause marrow and soft tissue edema and can be impossible to distinguish from edema due to infection. MRI can be limited by artifact related to metallic hardware that can obscure the surrounding tissues. While patients with orthopedic hardware can usually be imaged, assessment of the area immediately surrounding metallic hardware is frequently limited by distortion of the local magnetic field. The extent of metal susceptibility artifact varies with the size and type of metal and can be minimized using certain imaging sequences (e.g., high-resolution fast or turbo spin echo sequences). New MR imaging techniques promise even more robust metal suppression, with the potential ability to image tissues immediately adjacent to metal, but these are not yet widely available [89]. Susceptibility artifact is generally more pronounced with stainless steel and less pronounced with titanium. Some, but not all, external fixation devices are MR-compatible. Some are ferromagnetic or paramagnetic and might displace in the magnetic field, so external fixation constructs must be tested for magnetic susceptibility prior to imaging. Moreover, any metal implant can result in local tissue heating, so patients with metal implants must be able to sense and communicate discomfort to the MR technologist at the time of imaging. MRI is contraindicated in patients who have pacemakers and other electronic implants, ferromagnetic cranial aneurysm clips, and intra-ocular metal. Some MRI-compatible versions of spinal stimulators and pacemakers have recently been developed, but these are not yet in common use [90]. Most claustrophobic patients can be imaged with sedation or with the use of an open architecture magnet. The current generation of MRI machines, even when not formally described as "open" magnets, are built with shorter, wider bores ("tubes") and are often well tolerated. Weight limitations for obese patients currently range from 300 to 450 pounds, depending on the magnet.

Table 5.3 Indications for MRI in detection of infection

Characterize soft tissue abnormalities
Exclude osteomyelitis
Preoperative assessment

Table 5.4 MRI sequences—characteristics and applications

Sequence	Parameters	Use	Characteristics
T1-weighted (Fig. 5.14)	Short TE Short TR	Good for demonstrating anatomy	Normal fat and fatty marrow is bright or hyperintense on T1-weighted images
Proton density-weighted	Short TE Long TR	Good for demonstrating anatomy	Similar to T1-weighted sequence, but fluid and muscle are not as dark or low signal
T2-weighted	Long TE Long TR	Fluid-sensitive	Fluid and edema are bright or hyperintense on T2-weighted images, but may be hard to distinguish from fat, unless fat saturation is employed
Fat-saturated T2-weighted	Long TE Long TR	Fluid-sensitive (very)	Fluid and edema are bright or hyperintense; fat is dark or hypointense
	Fat saturation pulse		Very sensitive screen for fluid collections and for edema associated with infection or inflammation
STIR (Fig. 5.14)	Long TR Intermediate to long TE	Fluid-sensitive (very)	Normal fatty marrow is dark or low signal. Edema and fluid collections become bright or high signal
	Inversion recovery pulse		Very sensitive screen for fluid collections and for edema associated with infection or inflammation, but anatomic detail is not well depicted
Fat-saturated proton density-weighted	Short TE Long TR	Fluid-sensitive	Normal fatty marrow is dark or low signal. Edema and fluid collections become bright or high signal
	Fat saturation Pulse		Can also screen for fluid and edema
T1-weighted with fat saturation ("fat sat") (Fig. 5.13)	Short TE Short TR	Gadolinium contrast sensitive	Gadolinium contrast appears bright or high signal. Abscesses and proteinaceous or hemorrhagic fluid can also appear bright/high signal. Fat and simple fluid are dark or low signal
	Fat saturation pulse		Obtained both before and after IV contrast to detect contrast enhancement. Pre- and post-contrast sequences can be compared visually or computationally subtracted to demonstrate enhancing areas. Inhomogeneous fat suppression can occur, particularly in the foot, and should not be mistaken for enhancement When used without contrast, this sequence can distinguish fatty masses from hemorrhagic or proteinaceous fluid, e.g., lipoma from hematoma or proteinaceous ganglion cyst

MRI scanners produce images using a strong magnetic field and radiofrequency (RF) waves. The magnetic field creates an equilibrium state for the atoms in the body, the RF wave perturbs the atoms, and the scanner then records how different atoms respond. Clinical magnets range in field strength from 0.2 Tesla to 3 Tesla: the higher the field strength, the higher the potential signal-to-noise and spatial resolution (anatomic detail) in the resultant images. A variety of open, wide-bore, short-bore, and dedicated extremity magnets are now available. In order to optimally detect the signal produced by tissues in response to the radiofrequency wave perturbation and to generate high-resolution images, local RF receiver coils ("coils") are employed. Thus, for imaging the foot, a small diameter tubular extremity or foot-and-ankle coil is placed around the extremity. A typical MRI exam lasts 30–60 min, during which time approximately 4–8 imaging sequences are acquired. A sequence is a set of images designed to highlight specific tissue features and can be obtained in axial, coronal, sagittal, or any desired orientation. Some newer systems can obtain a 3D sequence that can then be reformatted into any plane. Imaging sequences are described in terms of the length of their TR (time-to-repetition) and TE (time-to-echo) times and in terms of any special radiofrequency pulses they employ (e.g., fat saturation or inversion recovery pulses). Commonly used imaging sequences are reviewed in Table 5.4. Anatomic and pathologic structures are described in terms of their signal intensity on a specific imaging sequence, often in relation to muscle. For example, fat and fatty marrow appear bright on T1-weighted images and are described as hyperintense or high signal intensity on T1-weighted images. They are low signal on fat-saturated T2-weighted and STIR sequences and are described as hypointense or low signal intensity. In contrast, simple fluid or edema is hypointense on T1-weighted images and hyperintense on T2-weighted, fat-saturated T2-weighted, and STIR sequences. Because gadolinium contrast and fat are both bright on T1-weighted images, contrast-enhanced images are often obtained using fat saturation techniques, so that fat appears darker and gadolinium contrast is bright. This is particularly useful in the foot where fatty marrow predominates. Nonetheless, it can be challenging to achieve homogeneous fat saturation in the foot which, in turn, makes it difficult to evaluate for the presence of absence of contrast enhancement [91]. Optimal

images are acquired by maximizing image signal-to-noise and using it to achieve high spatial resolution, based on appropriately small fields of view, thin slices, and smaller imaging voxel sizes. However, imaging at high spatial resolution requires longer imaging times.

Unlike CT, MRI provides high intrinsic soft tissue contrast, without the use of intravenous contrast agents. As a result, exogenous intravenous (IV) contrast is not required in order to detect changes of soft tissue infection or osteomyelitis—these processes appear as abnormal edema-like signal in the soft tissues and bones, respectively. However, IV contrast can play a role in imaging of infection in the diabetic foot by delineating soft tissue and intraosseous abscesses, highlighting fistulous tracts between ulcers and bone, and facilitating MR angiography. Gadolinium concentrates in areas of infectious or noninfectious inflammation, because of both increased vascularity and increased "porosity" of arteries in those settings and produces hyperintense (bright) signal on T1-weighted images.

Most contrast agents employed for clinical MR imaging are based on the paramagnetic element gadolinium. Historically, gadolinium contrast has been better tolerated than the iodinated forms of contrast used for CT scans and catheter angiography, with lower risks of anaphylactic reactions and lower risk of nephrotoxicity. However, recently, gadolinium-based contrast media have been linked to the disease nephrogenic systemic fibrosis (NSF) in patients with severely impaired renal function [92, 93]. Nephrogenic systemic fibrosis (NSF), formerly known as nephrogenic fibrosing dermopathy, is a disfiguring and potentially disabling or fatal disorder, characterized by symmetric, coalescing, indurated skin plaques, that can also cause joint contractures and fibrosis in internal organs. The link between intravenous gadolinium contrast and NSF is stronger for certain gadolinium formulations and seems to be dose-related [62]. Of note, follow-up dialysis after administration of gadolinium contrast does not appear to prevent NSF [93, 95]. Having noted that, in patients with end-stage disease on chronic dialysis, when gadolinium administration cannot be avoided, the ACR Committee on Drugs and Contrast Media recommends that MRI examinations performed with gadolinium-based contrast agents be performed as closely before hemodialysis as is possible, "as prompt post-procedural hemodialysis, although unproven to date, may reduce the likelihood that NSF will develop" [64]. Due to concerns over NSF, the Federal Drug Administration (FDA) now recommends screening patients prior to administration of a gadolinium-based contrast agent to identify individuals with acute or severe chronic renal insufficiency. In our institution, this assessment is made in the MRI department, prior to contrast administration [94, 95]. Most recently, new concerns regarding gadolinium-based contrast agents have been raised by the observation that residual gadolinium accumulates in patients' brain and bone, even in patients with normal renal function, though the clinical significance of this finding remains to be determined [96]. A more thorough discussion of these topics is provided in the American College of Radiology Manual on Contrast Media [64].

Findings

On MR images, cellulitis appears as an ill-defined area in the subcutaneous fat that is of low signal on T1-weighted and high signal on STIR and T2-weighted sequences [17] (Fig. 5.2). It can be seen as both strand-like reticulated pattern of high T2 signal extending along septae between lobules of fat and as more confluent dense high T2 signal. However, this signal pattern is nonspecific and is common to both cellulitis and non-cellulitic edema. Gadolinium administration may identify uncomplicated cellulitis, which typically shows uniform enhancement of subcutaneous edema [16].

Abscess presents as a focal lesion that is low signal on T1-weighted images and high signal on T2-weighted and STIR images. Without intravenous gadolinium, an abscess may not be distinguishable from dense soft tissue edema seen in severe cellulitis or from soft tissue phlegmon [97]. Following administration of intravenous gadolinium, an abscess demonstrates peripheral or rim enhancement, demarcating the fluid collection within (Fig. 5.13). The enhancing rim is believed to correspond to granulation tissue in the pseudocapsule. However, rim enhancement is a sensitive but nonspecific sign for abscess and can be seen in necrotic tumors, seromas, ruptured popliteal cysts, and hematomas [97]. Pus in the center of the abscess can have variable signal intensity, depending on its contents. Simple fluid will have low T1/high T2 signal, but abscesses often have high T1 signal content due to the presence of proteinaceous material within the fluid. Like proteinaceous fluid, hemorrhage can also appear high signal on T1-weighted images. Because this high T1 signal intensity appearance could be mistaken for gadolinium enhancement, comparison of pre- and post-contrast images becomes essential.

The diagnosis of septic arthritis is generally made clinically and confirmed by percutaneous joint aspiration or surgery [16]. The MR appearance of septic arthritis consists of joint effusion, often with synovial thickening, intra-articular debris, and surrounding reactive marrow and soft tissue edema. Following administration of intravenous gadolinium, there is intense synovial enhancement. Periarticular reactive marrow edema may demonstrate gadolinium enhancement even in the absence of osteomyelitis [16]. This constellation of findings is suggestive, but not specific for, infection and can also be seen in inflammatory conditions such as rheumatoid arthritis and seronegative arthropathies.

The primary MRI finding in osteomyelitis is abnormal marrow signal that enhances [97]. The abnormal marrow appears low signal (dark) on T1-weighted images and high

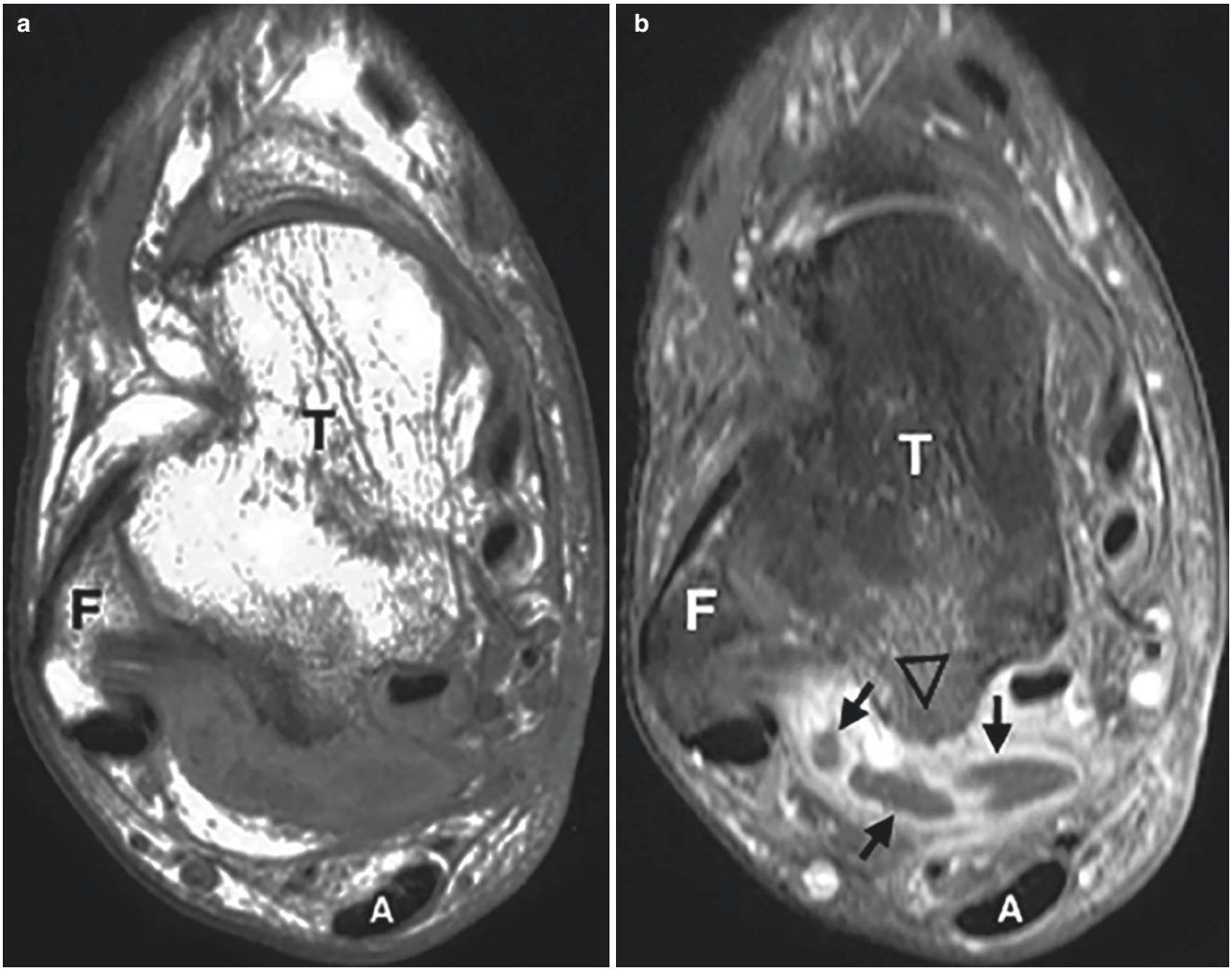

Fig. 5.13 Soft Tissue Abscess on MRI. Axial images of the ankle in a diabetic patient with ankle swelling. T1-weighted image (**a**) shows abnormal low signal posterior to the talus. Fat suppressed T1-weighted image (**b**) was obtained after IV administration of gadolinium. Note the bright enhancing peripheral rim (arrows) surrounding the abscess. The rim is slightly thickened. Central non-enhancement confirms fluid content. Enhancement is also seen in the adjoining portion of the talus and the intervening talar cortex is thinned and irregular (open arrowhead). Because they abut the abscess, these findings in the bone are highly suggestive of osteomyelitis. *A* Achilles, *F* fibula, *T* talus

signal (bright) on fluid-sensitive images such as fat-saturated T2-weighted and STIR images, typically with ill-defined margins (Fig. 5.1) (Table 5.5). Changes in marrow signal intensity can be detected as early as 1–2 days after onset of infection [33, 98]. Following intravenous administration of gadolinium contrast, the abnormal marrow enhances and is seen as a bright area on the fat suppressed T1-weighted images. Secondary signs of osteomyelitis include cortical interruption, periostitis (seen as enhancement at the margins of the periosteum) and a cutaneous ulcer or sinus tract in contiguity with the abnormal marrow [16, 99]. Contrast does not identify new areas of signal abnormality compared with fat-saturated T2-weighted or STIR sequences [98]. Rather it helps demonstrate soft tissue and intraosseous abscesses and outline fistulous tracts between osteomyelitis and the skin [98]. It can also distinguish joint fluid from thickened synovium. Morrison et al. reported improved sensitivity and specificity for detection of osteomyelitis, using gadolinium

Table 5.5 MRI findings of osteomyelitis

Primary signs
Hyperintense (bright) marrow signal on STIR sequence
Hypointense (dark) marrow signal on T1-weighted sequence
Enhancing marrow on post-contrast T1-weighted sequence
Secondary MR signs
Periosteal reaction
Subperiosteal abscess
Periostitis (manifested by periosteal enhancement)
Cortical destruction
Ulcer
Sinus tract

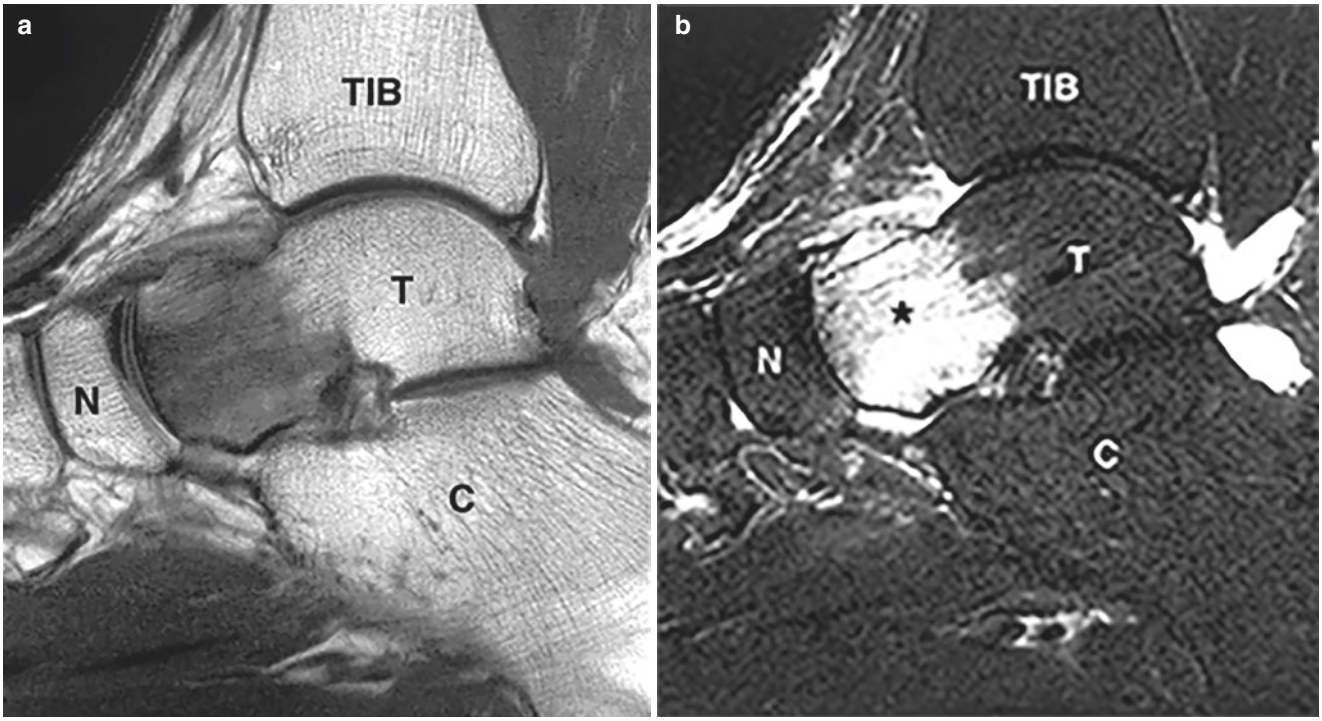

Fig. 5.14 Marrow Edema on MRI. Sagittal images of the ankle show marrow edema (*) which is (**a**) dark on T1-weighted image and (**b**) bright on STIR images. This marrow edema pattern is nonspecific and is similar to the marrow changes in osteomyelitis. However, this patient sustained trauma to the anterior talus and, here, the marrow edema represents a bone bruise. Specificity and accuracy can be improved by administration of gadolinium, as osteomyelitis frequently shows marrow enhancement. *C* calcaneus, *N* navicula, *T* talus, *TIB* tibia

contrast—88% sensitivity and 93% specificity for contrast-enhanced studies versus 79% sensitivity and 53% specificity for non-contrast-enhanced images [97]. Sensitivity and specificity of various secondary signs for identifying osteomyelitis were: sinus tracts (32/85%), cellulitis (84/30%), soft tissue abscess (26/74%), ulcers (41/81%), cortical tract or disruption (86/78%) [99]. A negative MRI effectively excludes osteomyelitis [100].

The sensitivity and specificity of MRI for detection of osteomyelitis compiled from five studies is 96% and 87% respectively [72, 101–104]. Sensitivities and specificities for detection of osteomeylitis in diabetic individuals are lower, respectively, 82% and 80%, in large part due to neuroarthopathic changes [97, 105]. Ahmadi et al. identified features that can help to distinguish between osteomyelitis and neuropathic arthropathy [106]. They examined 128 neuropathic joints in 63 patients and concluded that features more indicative of infection were sinus tract, replacement of soft tissue fat, fluid collection, or extensive marrow abnormality, while features indicative of neuroarthropathy without infection were a thin rim of peripheral enhancement around an effusion, the presence of subchondral cysts, or the presence of intra-articular loose bodies.

Because of its high negative predictive value, MRI can facilitate accurate depiction of the maximum possible extent of marrow involvement by osteomyelitis. As such, MRI can help for planning of foot-sparing surgical procedures [97]. Marrow involvement is well-demonstrated on fluid-sensitive images, such as fat-saturated T2-weighted or STIR sequences.

Its advantages notwithstanding, MRI has several important limitations. MRI of the infected diabetic foot yields a significant number of false-positive diagnoses. The kind of abnormal marrow signal associated with osteomyelitis can also be seen with neuroarthropathy, including silent bone stress injuries associated with diabetic neuroarthropathy, bone contusions, fractures (Figs. 5.14 and 5.15), and, occasionally, osteonecrosis. The hyperemic phase of osteoarthropathy may display enhancing marrow edema indistinguishable from osteomyelitis. Intense soft tissue inflammation may also give rise to reactive edema in the adjoining bone, in the absence of osteomyelitis. False-negative contrast enhancement can occur in the setting of vascular insufficiency [107]. The utility of MR imaging for following response to treatment of osteomyelitis remains to be defined. Due to its high sensitivity for detection of soft tissue and marrow edema, MRI findings can be expected to lag behind the clinical response in treatment of soft tissue infection and osteomyelitis. As noted above, the use of gadolinium contrast in patients with severe renal failure is now generally contraindicated.

In addition to assessment of bone and soft tissue infection, there is great interest in the use of anatomic MRI [108–

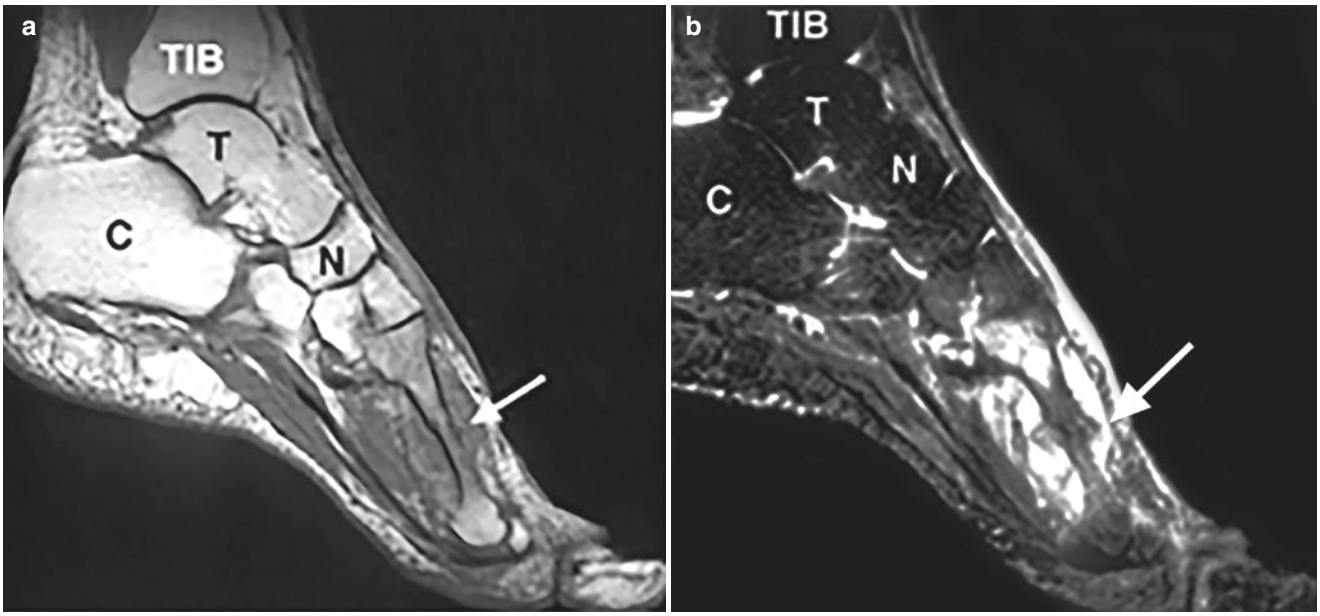

Fig. 5.15 Stress fracture on MRI. Sagittal T1-weighted (**a**) and STIR (**b**) MR images of the foot demonstrate cortical irregularity of the mid-diaphysis of the metatarsal bone (arrow). The marrow signal is abnormal, consistent with a marrow edema pattern: low signal on the T1-weighted image and high signal on the fluid-sensitive STIR image. The fracture line (arrow) remains dark on both sequences and is surrounded by bright edematous marrow on the STIR image. Marked soft tissue swelling surrounding the fracture is also better appreciated on the STIR images (**b**). *C* calcaneus, *N* navicular, *TIB* tibia, *T* talus

110], MR spectroscopy [111–113], and MR elastography [114] to identify early changes of structural and metabolic pathology in the diabetic foot.

Angiography

Angiography is indicated in diabetic patients with nonhealing ulcers or osteomyelitis who require mapping of vascular disease prior to endovascular or surgical treatment. Almost without exception, patients with nonhealing foot ulcers will have severe steno-occlusive disease involving all three runoff vessels of the calf (anterior tibial, posterior tibial, and peroneal arteries). In this patient population, 20% of peripheral bypass grafts will have to extend to a pedal artery. The distal anastamosis is either to the dorsalis pedis artery or to the proximal common plantar artery trunk [115]. Thus, detailed mapping of arterial disease from the abdominal aorta to the pedal vessels is necessary.

Several alternative—and, in some cases, complementary—techniques currently exist for mapping the vessels in the diabetic foot: conventional catheter angiography (CA) and digital subtraction angiography (DSA), MR angiography (MRA), CT angiography (CTA), and duplex Doppler ultrasound (DU). These techniques are reviewed below. In general, vascular disease in diabetics tends to predilect the smaller caliber vessels of the distal lower extremity, which poses special challenges for imaging.

In brief, digital subtraction angiography DSA historically has been the imaging gold standard because of superior spatial and temporal resolution. However, with the progressive improvement of noninvasive techniques, DSA as a routine diagnostic technique is being supplanted by noninvasive cross-sectional techniques, with catheter angiography often being reserved for cases where percutaneous intervention is planned [116, 117].

Catheter Angiography: Conventional and Digital Subtraction Angiography (CA, DSA)

Traditionally, vascular imaging has been performed using conventional angiography [118]. Conventional angiography is an invasive procedure, performed in the angiographic suite under fluoroscopic (real-time X-ray imaging) guidance. A thin, flexible catheter is inserted into the aorta or arteries, usually via a femoral artery approach. A relatively large bolus of iodinated contrast is injected into the intraluminal catheter and rapid sequence radiographs are exposed. Although examination of the abdominal aorta and iliac vessels can readily be performed with a multi-sidehole catheter in the abdominal aorta, examination of the femoral, popliteal, tibioperoneal, and pedal arteries entails placement of a catheter in the ipsilateral external iliac artery. Selective catheter placement has the advantage of limiting contrast burden in a patient group predisposed to renal insufficiency.

Digital subtraction angiography (DSA) has replaced the older form of hardcopy, cut-film angiography in most

institutions [62]. DSA is particularly advantageous for imaging diabetic arterial disease, because it is superior in terms of demonstrating small caliber distal vessels and uses less contrast to do so. In DSA, a set of images of the limb is obtained prior to administration of contrast (known as a "mask") and stored electronically. AP and lateral images are then obtained during administration of contrast, along the length of the vessels of interest, including one perpendicular to the interosseous membrane, that separates out the anterior tibial and peroneal vessels. Pre- and post-contrast image sets are subsequently subtracted by the computer to generate a final DSA image set that shows the intra-arterial contrast map (Fig. 5.16). Using DSA, the interventionalist can perform rapid road-mapping of the vasculature during a procedure, without having to wait for hardcopy films to be developed. Nonionic iso-osmolar contrast agents, although more expensive, are typically used because they are associated with less pain and a lower risk of contrast-induced nephropathy, a risk

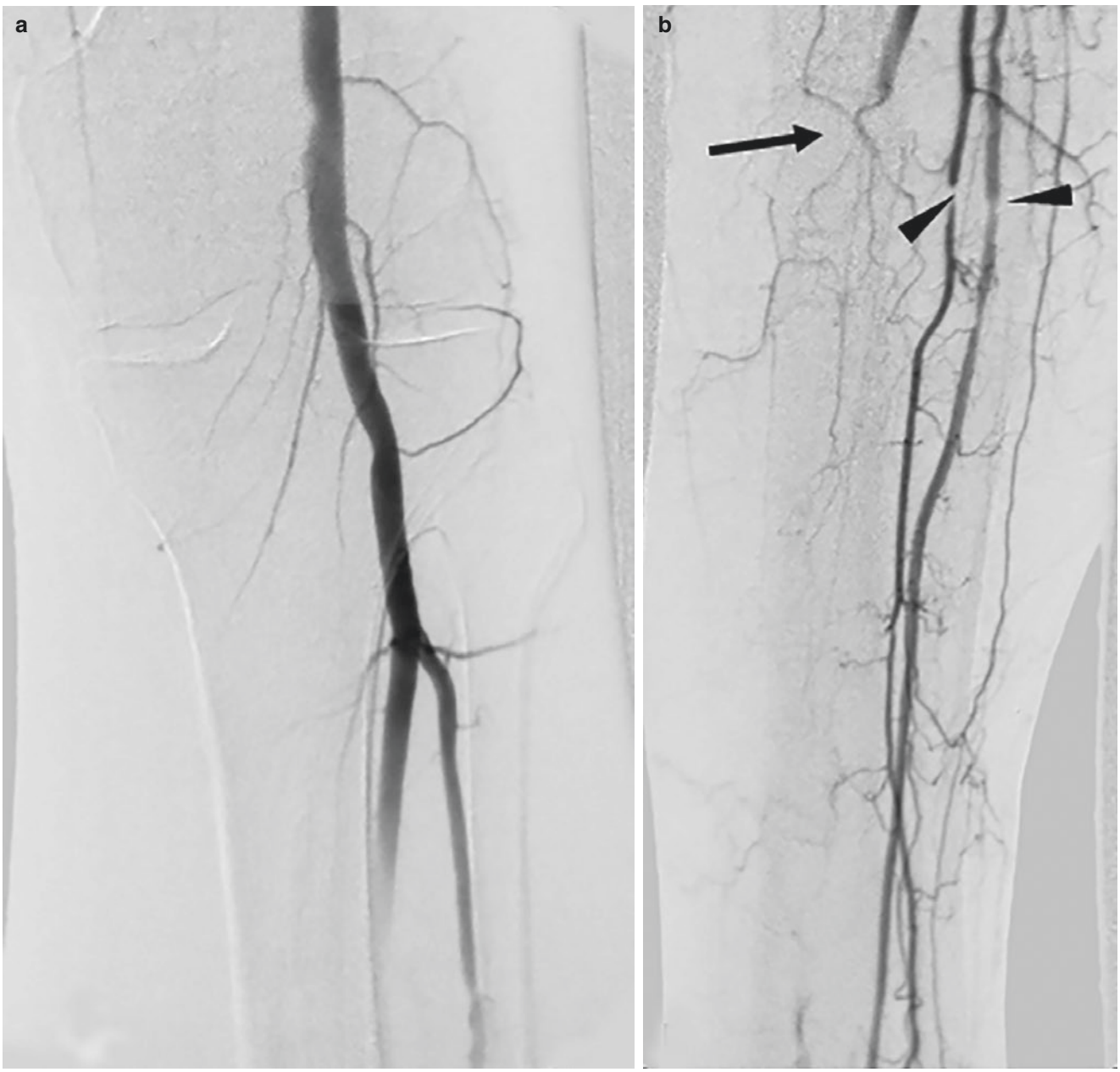

Fig. 5.16 Digital subtraction angiogram (DSA) of the lower extremity in 74 years old diabetic man with nonhealing heel ulcer. (**a**) Images demonstrate a patent popliteal artery, anterior tibial artery, and tibioperoneal trunk. (**b**) The posterior tibial artery is occluded (arrow) and there are stenoses in the proximal anterior tibial and peroneal arteries. (**c**) Just above the ankle, the left peroneal artery is occluded and there is reconstitution of a short diseased posterior tibial artery (bracket) from collateral vessels. The dorsalis pedis (DP) artery is patent (arrow). (**d**, **e**) Images in the foot show patent DP (arrows)

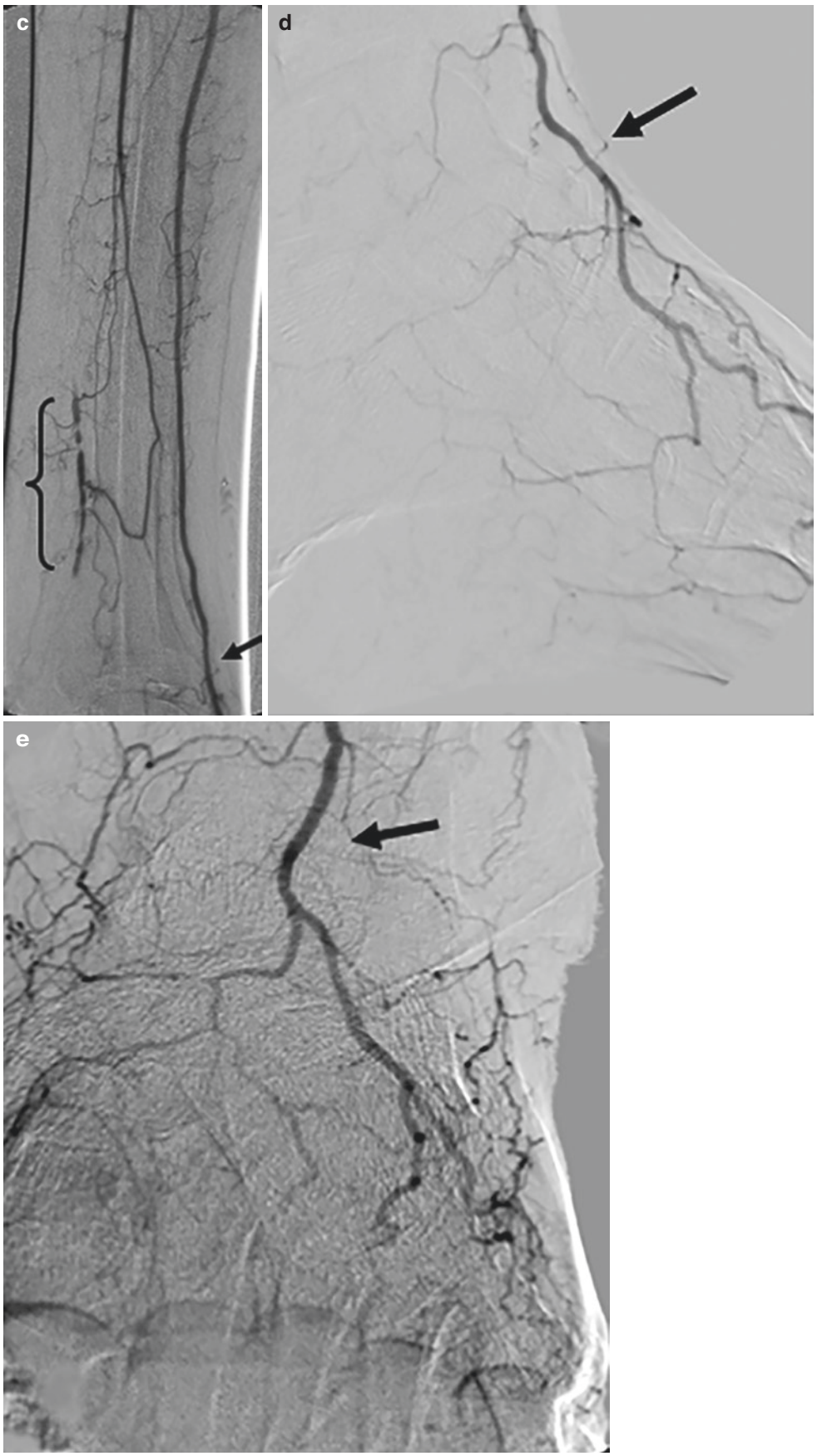

Fig. 5.16 (continued)

that is higher in diabetic patients [119]. Newer high-resolution flat panel image intensifiers can cover larger fields of view and facilitate fewer injections and decreased radiation exposure. Portable and surgical suite DSA systems are available.

Conventional angiography, including DSA, remains the gold standard for arteriographic imaging. The major advantage of conventional angiography is that it provides access to perform not only diagnostic, but also therapeutic, vascular procedures, including angioplasty, atherectomy, stenting, and thrombolysis. A well-timed study can provide very high spatial resolution images of small vessels. The major risks of catheter-based angiography include radiation exposure, potential for bleeding, injury to the vessel wall, dislodgment of embolic material, and risk of renal failure or allergic reaction from the iodinated contrast. Injury to the femoral artery access site can be decreased with use of lower profile catheters and sheaths and the use of ultrasound-guidance for placing the catheter [62]. Not infrequently, vascular disease and slow flow can disrupt the timing of the exam, with resultant failure to demonstrate the distal vessels. This is especially problematic when demonstration of distal vessels is the key to planning a bypass graft procedure. Good technique is key for successful opacification of the distal tibial and pedal arteries.

There are several strategies for reducing contrast exposure related to catheter angiography in patients with renal insufficiency: (1) if the femoral pulse is normal, a choice may be made to limit angiographic imaging to the extremity itself, and forgo examination of the aortoiliac arteries; (2) the catheter can be advanced distally, into the distal superficial femoral or popliteal artery, for the injection, instead of performing the injection proximally, in the external iliac artery; (3) full strength contrast can be diluted with normal saline; (4) carbon dioxide (CO_2) can be used, instead of iodinated contrast, for examination of the aorta and pelvis [62].

Because DSA is performed using invasive technique, ionizing radiation, and iodinated contrast agents and because it is considered relatively intensive in terms of expense, labor and time, DSA is now generally reserved for guidance of endovascular intervention rather than for initial diagnosis [116, 120].

MR Angiography (MRA)

More recently, MRI has come to play a role in the imaging of arterial disease, in the form of MR angiography (MRA). MRA has the benefit of providing detailed anatomic mapping of arterial disease while, at the same time, obviating the need for arterial catheter placement and associated complications. Contrast-enhanced MRA (CE-MRA) and non-contrast-enhanced time-of-flight (TOF) MRA are the most commonly used techniques for performing MRA of the lower extremity [121, 122] (Figs. 5.17 and 5.18). Phase-contrast MRA, an alternative non-contrast-enhanced MRA technique, has not been in common use, but is being currently being revisited and new non-contrast-enhanced techniques are also being

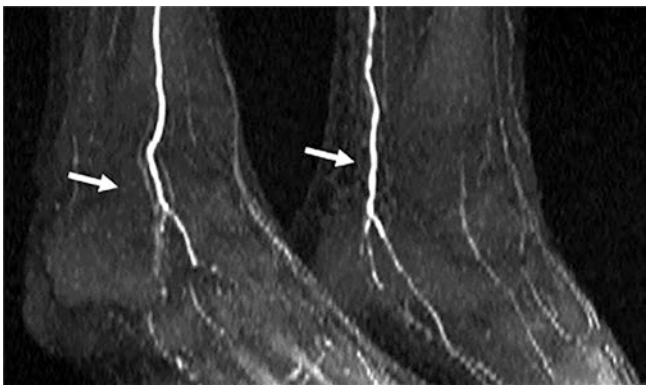

Fig. 5.17 Time-of-flight MR Angiogram in the ankle and foot demonstrates single-vessel run-off, with patency of the posterior tibial artery and portions of the plantar arteries on both sides (arrows)

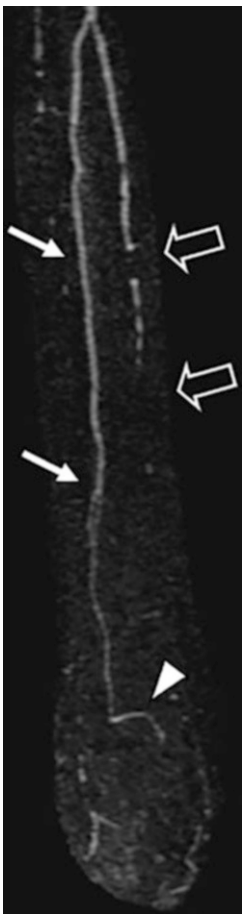

Fig. 5.18 Contrast-enhanced MRA for nonhealing ulcer. There is single-vessel runoff via the peroneal artery (arrow), to the level of the ankle joint, with reconstitution of an attenuated dorsalis pedis artery (arrowhead) via collaterals. The proximal posterior tibial artery demonstrates multiple stenoses and is occluded in the mid-calf (open arrow)

developed. Gadolinium, the contrast agent used in MRA, has traditionally been favored over the iodinated contrast used for catheter angiography, because of a lower incidence of allergic reaction and contrast-induced nephrotoxicity. However, as discussed above, new concerns regarding an association between gadolinium administration in patients with renal failure and development of a disease called nephrogenic systemic fibrosis (NSF) have arisen [64]. Moreover, most recently, accumulation of residual gadolinium contrast in patients' brain and bone has been observed, even in patients with normal renal function, though the clinical significance of this finding is still under investigation [96].

Time-of-flight (TOF) MR angiography relies on a non-contrast-enhanced, flow-sensitive MR sequence. Computer post-processing of the MR data generates coronal, sagittal or oblique reconstructions that mimic the appearance of conventional angiograms. TOF MRA can be time consuming, requiring 1–2 h to cover the distance from the aortic bifurcation to the distal lower extremity. Cardiac gating of the MR images improves image quality, but lengthens exam time, especially when the patient has a cardiac arrhythmia or is on beta-blocker medication. TOF MRA images tend to exaggerate the degree of steno-occlusive disease and are prone to motion and metallic susceptibility artifact [123]. However, TOF has shown accuracy comparable to DSA below the knee [124, 125].

Gadolinium- or contrast-enhanced MRA (CE-MRA) relies on intravenous injection of a small volume of gadolinium contrast and rapid imaging that is timed to optimally follow the passage of the contrast bolus through the arteries. This technique has the advantage of short scan time, reduced motion, and reduced susceptibility artifacts. It is more accurate than TOF MRA exam in depicting the grade of steno-occlusive disease and offers higher resolution in the distal arteries of the lower extremity [126, 127]. CE-MRA uses a much smaller volume of contrast than conventional angiography and therefore generates a smaller osmotic load and subsequently a lower incidence of nephrotoxicity. However, visualization of the arteries can be compromised by venous enhancement (Fig. 5.18) or by suboptimal arterial filling related to inaccurate timing of data acquisition. Use of rapid image data-sampling techniques for contrast-enhanced MRA, such as TRICKS (time-resolved imaging of contrast kinetics), can help with improve imaging of arteries in the foot. Specifically, these kinds of sequences help address problems with proper timing of the contrast bolus and reduced "venous contamination" of images, while improving conspicuity of small distal vessels [128].

In general, MR angiography achieves sensitivities of 92–97% and specificities of 89–98% [129, 130] and compares favorably to conventional angiography. Both TOF and CE MRA can reveal patent arteries not seen on conventional arteriograms, which can impact clinical decision-making [124, 131] 3D CE MRA is superior to 2D TOF MRA for detection and grading of peripheral arterial disease [131, 132]. Dorweiler et al. [133] examined the performance of pedal bypass grafts to foot vessels that were detected by magnetic resonance angiography (MRA), but occult at conventional angiography, in 15 patients with diabetes mellitus and severe arterial occlusive disease [133]. During 22-month mean follow-up, there was one perioperative graft occlusion and one major amputation, resulting in a secondary patency rate of 93.1% and a limb salvage rate of 89.5% at 36 months. Recent studies of contrast-enhanced MRA report sensitivity and specificity of >90% for detection of significant stenosis or occlusion [134, 135]. As with CTA, most contrast-enhanced MRA studies have been performed in patients with claudication, so accuracy for patients with critical lower limb ischemia is less well understood [116]. However, Owen et al. examined 30 patients with critical limb ischemia and found that diagnostic accuracy of contrast-enhanced MRA was similar to DSA [136]. The appropriate clinical role of MRA in the management of arterial disease in the diabetic foot is debated [137].

Because of concerns related to gadolinium administration in the setting of renal failure, new-generation techniques for producing non-contrast-enhanced MR angiograms are being explored and evaluated [138, 139]. For example, Hodnett et al. prospectively compared rapid unenhanced MRA (QISS or quiescent-interval single short technique) with contrast-enhanced MRA in 53 consecutive diabetic patients with symptomatic chronic lower limb ischemia and found that the diagnostic performance of nonenhanced MRA was nearly equivalent to contrast-enhanced MRA DSA [120, 138].

Computed Tomographic Angiography (CTA)

Lower extremity or peripheral computed tomographic angiography (CTA) is a relatively recent technique for evaluation of the peripheral arterial tree. With the advent of multidetector CT (MDCT) in 1998, CT imaging became fast enough to allow scanning of inflow and runoff vessels in the entire lower extremity, with sufficient spatial resolution, in a single CT acquisition. The acquisition time for these images is on the order of less than 1 min [131]. The minimum number of channels required to generate a peripheral CT angiogram is provided by a 4-detector scanner, but later generation 16- and 64-detector machines are preferred, because they provide near-isotropic 3D image sets, allowing reformatting of high-quality images in any plane [131, 140]. 64 detector CTAs are now in common use and allow examination of the entire peripheral vasculature with high diagnostic accuracy, yielding information on both the vessel lumen and the composition of the atheromatous plaque [141]. Images are generated using standard intravenous CT contrast, injected into an antecubital vein via power injector. Sophisticated scanning protocols are employed to optimize opacification in the arter-

ies of interest and the scanner table is moved during the scan to "chase" the contrast bolus [141]. As with conventional angiography, optimal timing of the contrast bolus is affected by cardiac function and by delays due to arterial pathology in the infrarenal aorta and lower extremity arteries. Venous enhancement may "contaminate" arteriograms when there is significant arteriovenous shunting or when longer scan times are used, but, with good technique, this should rarely pose a diagnostic problem [131]. Artifactual narrowing or occlusion of the dorsalis pedis artery ("ballerina sign") can occur with excessive plantar flexion of the foot, as it can with other forms of angiographic imaging [142]. CTA involves a relatively high radiation dose [143] and requires large volumes of contrast (150–180 CC) per run.

Once the initial CT angiographic images are acquired (Fig. 5.19a), the data associated with those images can be post-processed in order to generate clinically useful images (Fig. 5.19b–g), but this post-processing requires a high level of expertise, in order to avoid introducing post-processing artifacts that will degrade diagnostic accuracy. In some institutions, CT angiogram studies are post-processed by specially trained technologists in a dedicated image processing lab. Post-processing techniques include Maximum Intensity Projection (MIP) images, which mimic conventional angiography displays (Fig. 5.19b). These require subtraction of bone from the image, at which time there is a risk of inadvertently removing vessels adjacent to bone. Volume rendering (VR) represents a form of 3D surface display that does not rely on subtraction of bone from the image (Fig. 5.19c, d). In VR, however, vessels can be inadvertently removed by choice of VR parameters. In both MIP and VR techniques, stents and vessel calcifications can completely obscure the vessel lumen, making it difficult or impossible to assess flow in that segment—this can limit the utility of CTA in approximately 60% of patients with peripheral arterial occlusive disease [140]. In these cases, source images obtained perpendicular to the vessel can be useful. Curved planar reformations (CPR), which are longitudinal cross sections generated along a predefined vascular center line, can be generated along the length of the vessel, regardless of its course (Fig. 5.19e–g), but they require manual or semiautomated tracing of the vessel center line. With CPRs, artifacts mimicking vessel stenosis or occlusion can occur when the center line is not selected properly. When viewing CTA images, regardless of post-processing technique, care must be taken not to overestimate stenosis or occlusion due to artifactual "blooming" of calcifications or stents on narrow viewing windows. A viewing window of at least 1500 HU may be required [131]. Of note, when there is extensive vascular calcification in smaller crural or pedal arteries, it may be impossible to resolve the vessel lumen, notwithstanding proper window/level selection [131].

There is growing data available for assessment of the diagnostic accuracy of CTA in the evaluation of peripheral arterial occlusive disease. Reported sensitivity of CT angiography for detection of greater than 50% stenosis is on the order of 89–100%, with specificity ranging from 92 to 100% [138, 144]. Met et al. performed a meta-analysis of the diagnostic performance of CTA in peripheral arterial disease, compared with intra-arterial DSA, for grading disease severity in patients with PAD [145]. Based on 20 studies that met inclusion criteria and that yielded moderate methodological quality, they found overall sensitivity of CTA for detecting more than 50% stenosis or occlusion was 95% and specificity was 96%. CTA correctly identified occlusion in 94% of segments, the presence of more than 50% stenosis in 87% of segments, and the absence of significant stenosis in 96% of segments. Overstaging occurred in 8% of segments and understaging in 15%. They found corresponding improvement in diagnostic accuracy with increasing number of CT detectors (i.e., newer technology) [116, 145]. Diagnostic accuracy was lower for smaller distal vessels than for larger proximal vessels, but diagnostic performance below the knee remained good (sensitivity 85–99%, specificity 79–97%) [116, 145]. Interobserver agreement was good to excellent in most studies (k values>0.8) [116, 145]. Ota et al. compared cross-sectional imaging with DSA for assessment of lower extremity arterial occlusive disease and found that cross-sectional images generated by multidetector CTA demonstrated luminal cross-sectional area, whereas arterial stenosis on DSA, particularly when eccentric, showed poor correlation with reduction in cross-sectional area of the lumen, a key parameter in hemodynamic compromise [146].

Wilmann et al. examined the use of submillimeter collimated 16-detector MDCT in 39 patients and found sensitivity of 96% and specificity of 97%, even in popliteo-crural branches, using an effective radiation dose that was lower than for conventional DSA [147]. In a recent study of patients with critical limb ischemia ($n = 28$) using 16-detector CTA, treatment plans could be confidently formulated in 23 of the 28 patients [148]. The remaining five patients underwent supplementary DSA, but no DSA findings resulted in altered management. The authors found similar clinical utility of 16-detector CTA in patients with intermittent claudication [149]. To date, there is limited assessment of CTA for use in evaluating pedal arteries [150]. As CT technology progresses, availability of CT scanners with 256 multidetectors can be expected to provide more rapid, high-resolution imaging [151].

As suggested above, dense vascular calcification can potentially reduce diagnostic performance on MDCT [131, 152]. However, new CT technology, known as Dual Energy CT (DECT), may be able to play a role in improved plaque subtractions [153].

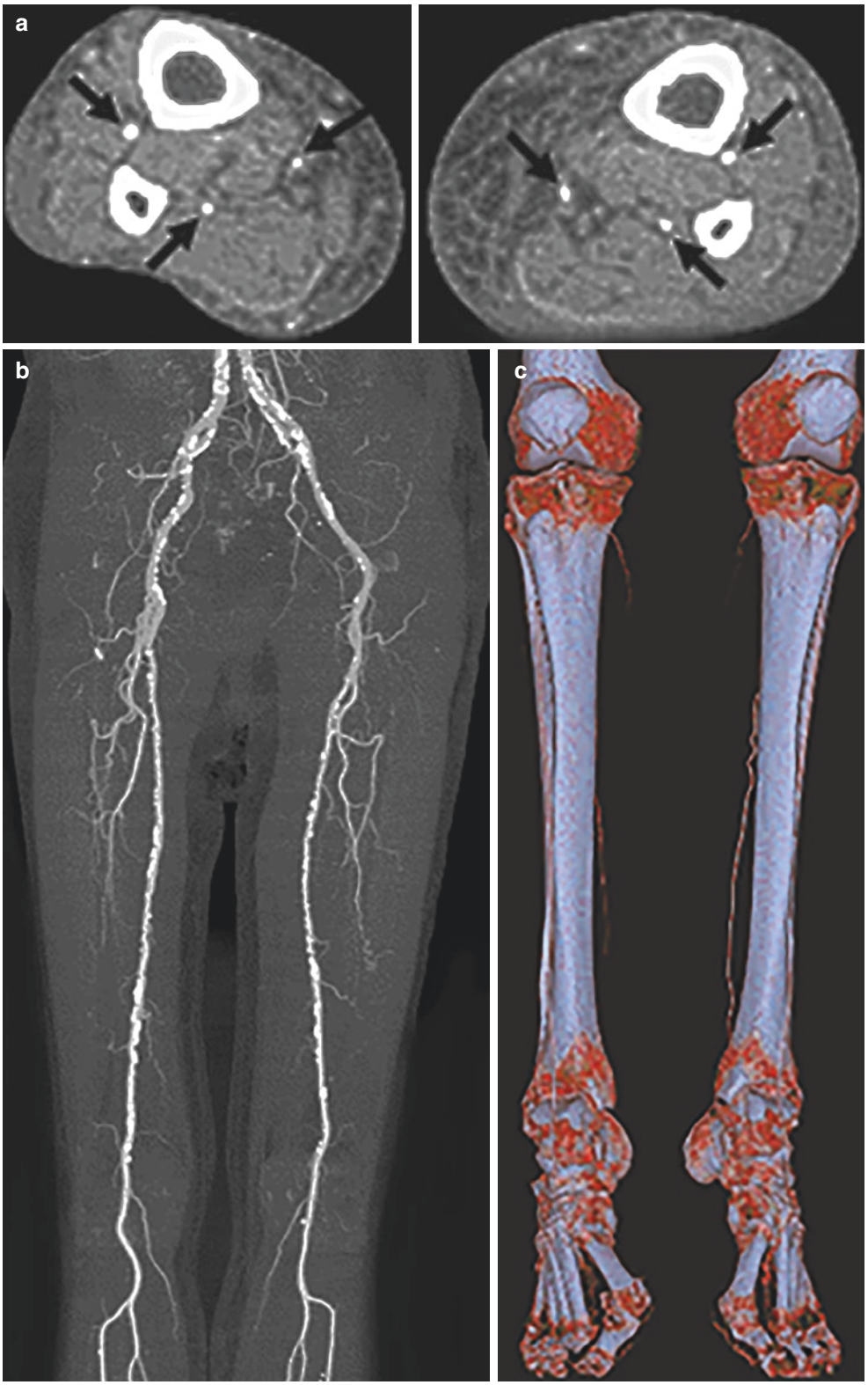

Fig. 5.19 CT angiogram with patent vessels in woman with concern for claudication. (**a**) Axial image of both lower extremities from a CT angiogram represents the source image for subsequent computer generated post-processed images. All three lower extremity run-off vessels are patent bilaterally, seen as small bright foci (arrows), due to administered contrast. (**b**) Maximum intensity projection (MIP) image was generated in the image processing lab from a stack of source images similar to (**a**) acquired through the lower body. The MIP mimics a conventional arteriographic display. Bilateral three vessel run off (proximal portion) is well depicted. Based on the protocol, images can be extended distally. Scattered areas of higher density (whiter) seen along the vessels reflects the presence of calcified atherosclerotic plaque. (**c**) AP and (**d**) oblique volume rendered (VR) images display the vessels in relation to bony anatomy, based on Hounsfield Unit density thresholds. (**e–g**) Curved planar reformatted images can be generated along the actual path of the vessel, in order to lay out the vessel in a single plane, respectively, depicting the anterior tibial, peroneal, and posterior tibial arteries

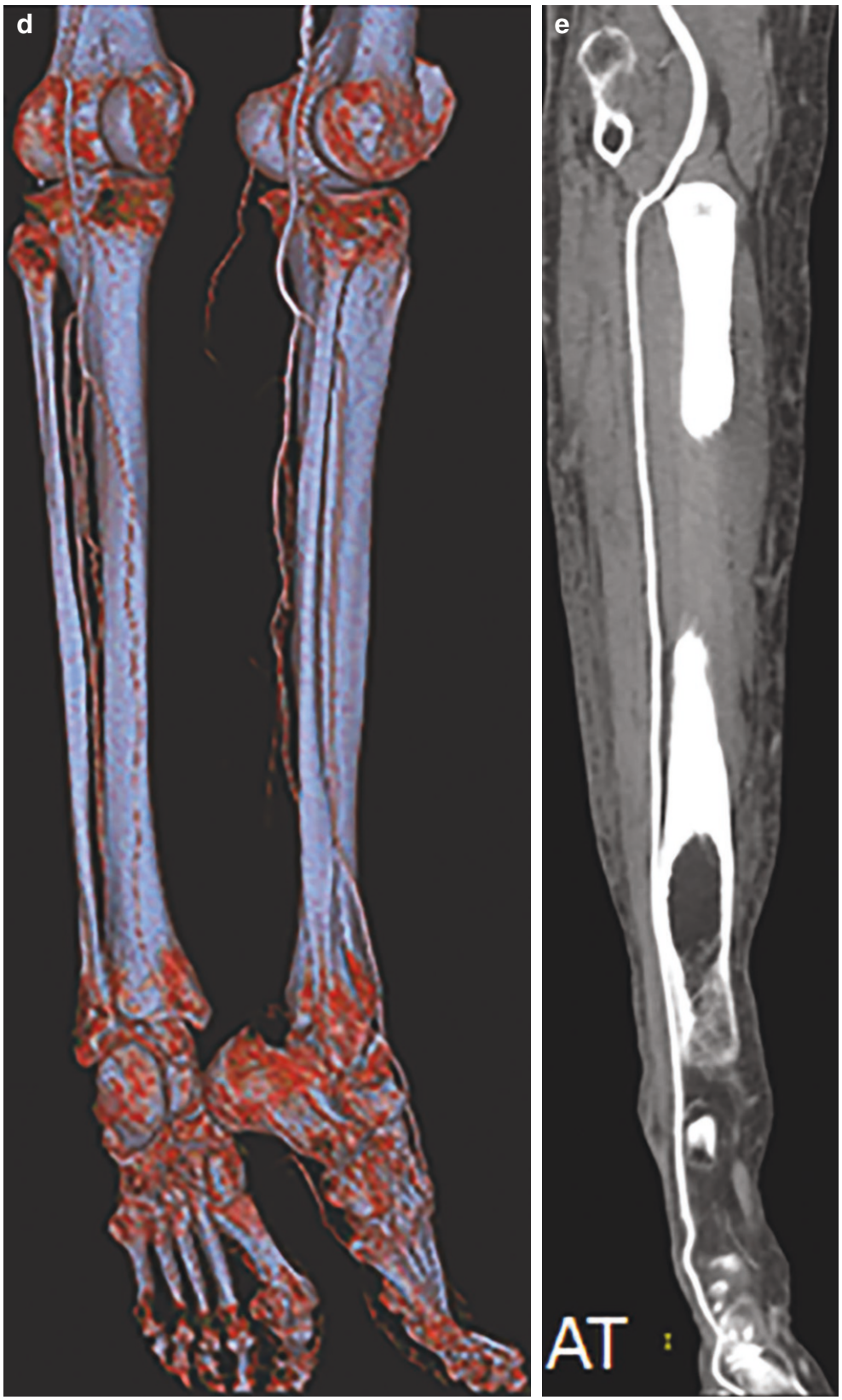

Fig. 5.19 (continued)

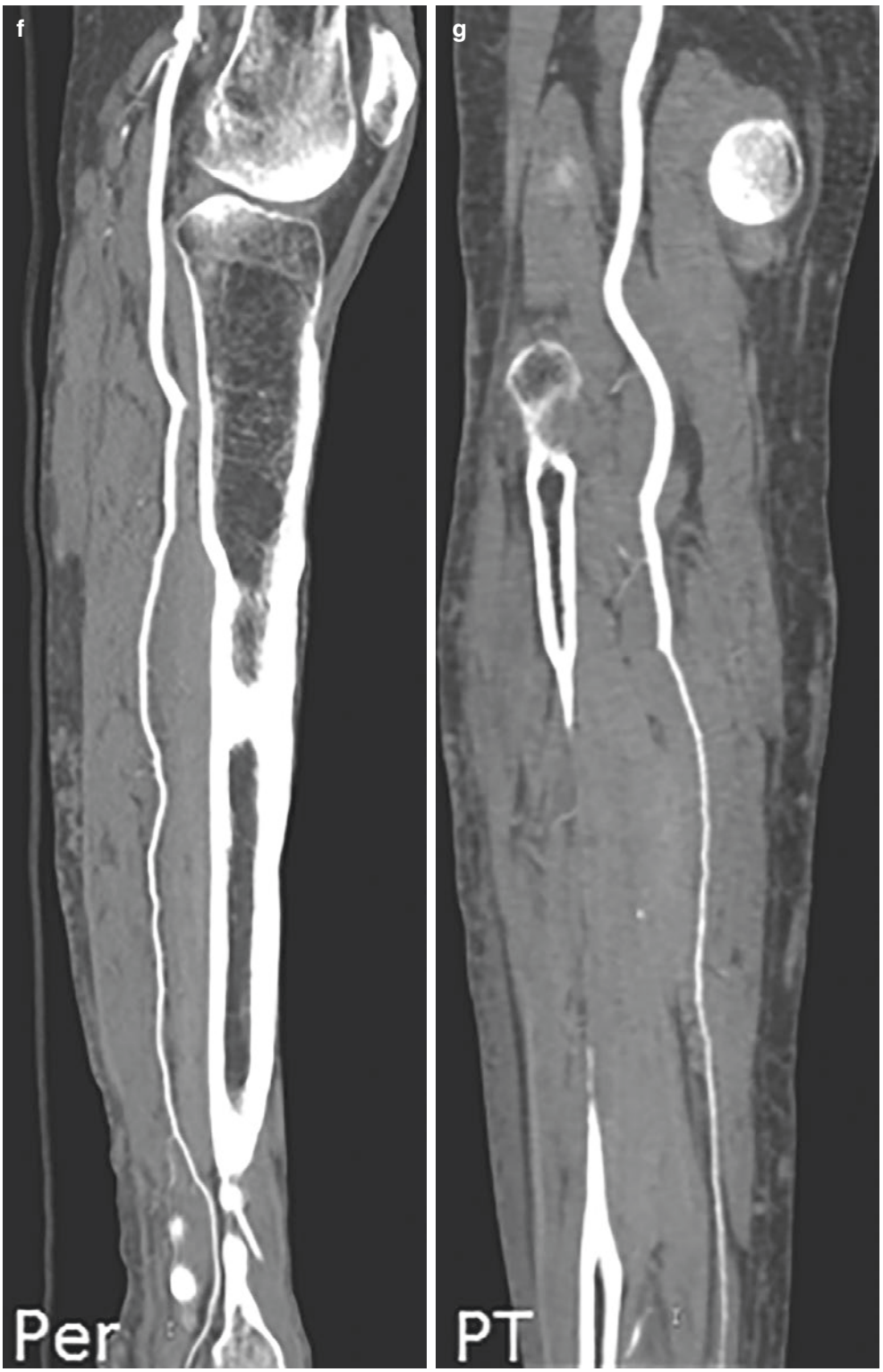

Fig. 5.19 (continued)

There is limited data on the diagnostic accuracy of CTA for quantification of in-stent restenosis in the lower limbs [154]. On CT images, stents are associated with artifact due to metal ("beam hardening" artifact) and in vitro studies have shown resultant over- and underestimation of stenosis on CTA [154]. Li et al. found overall sensitivity of 85% for detection of in-stent restenosis, but, 23.5% of stents were "unassessable," predominantly due to metal artifact [155].

Doppler Ultrasound

In addition to its ability to provide grayscale anatomic imaging, ultrasound can play an important role in depicting blood flow [156]. Three complementary techniques for blood flow imaging with ultrasound exist: (1) duplex Doppler ultrasound; (2) color flow imaging; and (3) power Doppler. These techniques are based on the Doppler effect: when a sound beam is reflected back off a moving object, the frequency of the sound beam is altered, increasing in frequency when the object (here, red blood cells) is moving toward the source of the sound beam, and decreasing when the object is moving away. The change in frequency is proportional to the velocity of the object and is greatest when the sound beam travels parallel to the vessel. Because Doppler measurements capture information about the velocity of blood flow, quantitative assessment of the severity of stenosis can be obtained, based on peak systolic and end-diastolic velocity measurements. Higher peak systolic measurements indicate more severe stenoses [157]. Using this technique, stenosis is graded as the ratio of peak systolic velocity of the target vessel divided by [the velocity in the adjacent non-stenosed vessel minus the peak systolic velocity ratio]. Findings are recorded on an anatomic diagram, creating a visual map of the vascular pathology. Doppler waveform analysis refers to depiction of the *pattern* of arterial blood flow, based on Doppler frequency shift. Patent arteries show a normal triphasic flow pattern. However, with increasing stenosis, the waveform flattens (Fig. 5.20). In duplex Doppler, the grayscale ultrasound image of the vessel and the vascular waveform are depicted together (Fig. 5.21). Duplex Doppler ultrasound can be used to image arteries and veins, to assess the severity and extent of peripheral artery disease, and to identify pedal arteries for bypass. Color Doppler images depict the frequency shift data as a color spectrum, that encodes both directional and velocity information. In color Doppler images, red and blue colors are superimposed on grayscale anatomic images of vessels, to indicate, respectively, flow toward and away from the transducer. Color Doppler images are often used in conjunction with duplex Doppler to aid in visualizing vessels. Doppler measurements and resultant images may be degraded by aliasing artifacts, either when the sampling frequency is too low or the angle of incidence between the sound beam and the vessel are too low. The third technique, power Doppler, is more sensitive to blood flow than color Doppler, allowing it to show smaller vessels and slower flow rates. Power Doppler scans assign color to flow, independent of its direction. Because of its high sensitivity, power Doppler can demonstrate flow associated with inflammation and neovascularity, such as inflammation associated with soft tissue infection and in soft tissues adjacent to osteomyelitis. Power Doppler can also help to distinguish between phlegmon and abscess, based on the lack of flow within the center of an abscess. With power Doppler, artifactual "flow" can occur with movement of the transducer or body part and false-positive and -negative findings can occur if the ultrasound machine's settings (color gain) are not properly set.

Many consider Duplex Doppler ultrasound (the combination of B mode and color Doppler ultrasound) to be the initial imaging modality of choice for evaluation of lower extremity arteries [117] because it is noninvasive and involves no ionizing radiation or contrast toxicity, is widely available and generally well tolerated by patients, and provides detailed hemodynamic information as well as potential to mark a target site. Ultrasound is also ostensibly less expensive than pre-procedure diagnostic angiography, though recent work by Ouwendijk et al. suggested that the true costs of DU may be more than expected, because of confidence levels and attendant need for supplementary imaging [158]. Disadvantages of Doppler ultrasound include: dependence of the exam on operator experience, relatively lengthy exam time, and limited ability to ensure that the entire area of interest has been imaged [131]. Moreover, DU examination can be difficult in the setting of tortuous vascular anatomy and/or overlying bowel gas in the abdomen and pelvis. In addition, mural calcification can cause acoustic shadowing and can also interfere with accurate measurement [159]. In practice, accurate Doppler measurements require a vascular laboratory with sufficient experience and attention to quality control. In the future, the use of ultrasound intravascular agents may contribute to improved imaging, but clinical utility of these techniques in the diabetic foot remains to be established [160].

Duplex ultrasound examination, performed in conjunction with clinical assessment, can and often is used for noninvasive preoperative planning of re-vascularization procedures in diabetic patients [116, 117, 159, 161, 162]. Ultrasound can be used to map occlusions for length and stenosis, based on velocity profiles [159]. For example, based on DU examination, increase in peak systolic velocity ratio >2 across a stenosis indicates reduction in cross-sectional caliber of >50% [116]. Though a complete discussion of the field is beyond this scope of this chapter, a number of studies have demonstrated the utility of duplex Doppler ultrasound in this setting. In a meta-analysis by Vissner et al., duplex US pooled sensitivity was 87.6% [95% CI: 84.4%, 90.8%] and pooled specificity was 94.7% (95%, CI: 93.2%, 96.2%). Compared with MR angiography, this represented a lower sensitivity and similar specificity. Doppler ultrasound interrogation can be performed with high degree of sensitivity and specificity in aortoiliac and femoropopliteal segments [161]. Duplex imaging of tibial vessels requires greater operator skill than imaging of larger, more proximal vessels, but can reliably identify stenosis and occluded segments and, in some cases, may be superior to angiography [163]. Hofmann et al. examined the use of preoperative high-

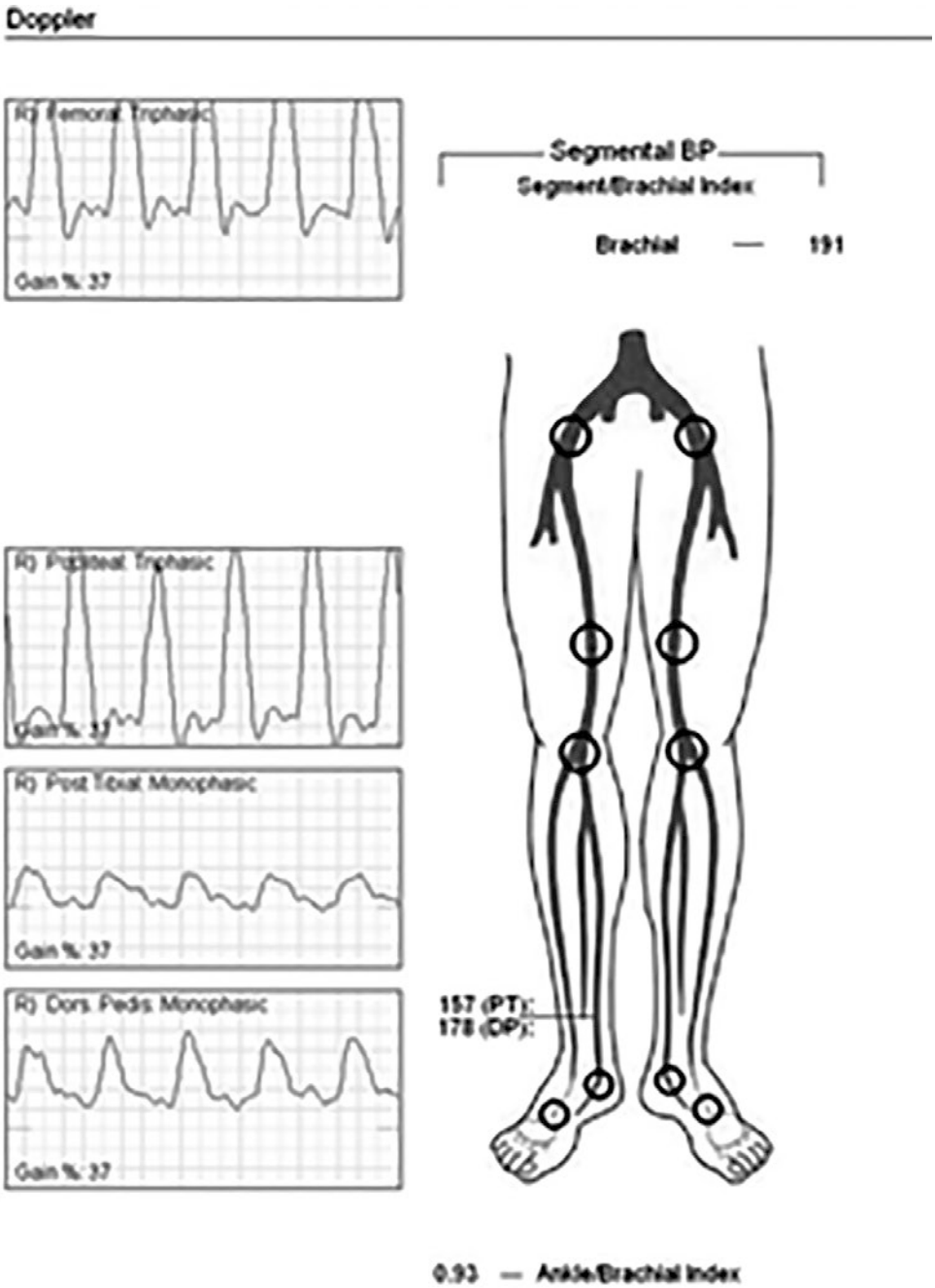

Fig. 5.20 Doppler waveform analysis in 62-year-old with right great toe ulcer and cellulitis. Arterial waveforms were evaluated using Doppler ultrasound at standardized sites along the ipsilateral lower extremity. While a normal triphasic wave pattern was observed in the femoral and popliteal vessels, a monophasic wave pattern was observed in the posterior tibial and dorsalis pedis vessels, indicating intervening stenosis

frequency duplex scanning of potential pedal target vessels [164]. They studied thirty-three consecutive diabetic patients suffering from critical limb ischemia, with indications of infra-popliteal occlusive disease, using a 13 MHz ultrasound probe, and attempted to identify the pedal target vessel best suited for surgery, based on inner diameter, degree of calcification, maximal systolic velocity, and resistive index. Results of Duplex scanning were compared with (1) results of selective digital subtraction arteriography (DSA) and contrast-enhanced MR angiography (MRA) interpreted by two radiologists; (2) the site of distal anastamosis predicted by a vascular surgeon based on the MRA and DSA; (3) the definitive side of distal anastamosis; and (4) early postoperative results. They found that Duplex scanning depicted significantly more pedal vascular segments than selective DSA, with relatively high agreement between the duplex ultrasound prediction and the definitive site of anastamosis (kappa 0.82). Levy et al. examined 105 consecutive lesions angioplastied among 56 patients undergoing 60 endovascular procedures, including aortoiliac, infra-inguinal and bypass graft lesions. Of these procedures, completely noninvasive evaluation was accomplished in 43

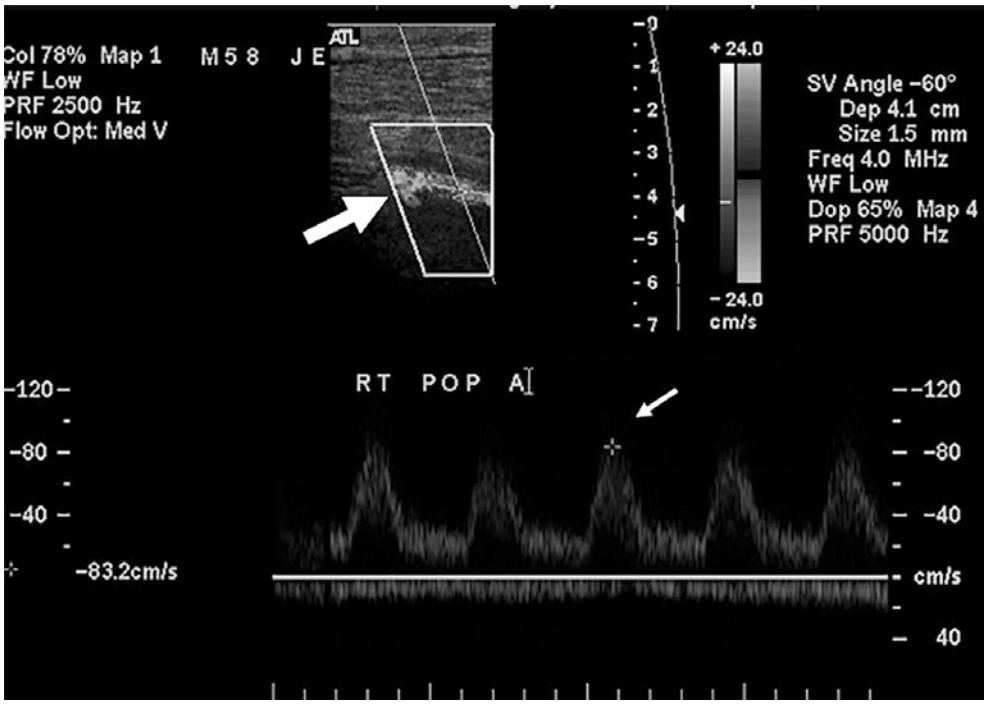

Fig. 5.21 Duplex Doppler examination at popliteal artery. The grayscale ultrasound image of the popliteal artery (thick arrow) is used to position the cursor for the measurement. Here, Color Doppler is being superimposed on the vessel to help highlight the artery and arterial flow velocities. The popliteal artery waveform generated by the measurement is shown below. A cursor is placed at the height of the waveform peak (thin arrow) and yields a peak flow rate of 83 cm/s, with no evidence of stenosis. Duplex Doppler ultrasound can be used to generate data like this along the length of a vessel, in order to map the site, length, and severity of stenoses

procedures (73%), either by means of duplex scanning ($n = 11$, 18%) or by means of MRA ($n = 32$, 53%) [165]. The findings at noninvasive exam were confirmed at intraoperative angiography and no additional lesions were identified. ABI and mean limb status category both showed significant improvement. The noninvasive approach was less expensive compared with pre-procedural contrast angiography, with $551 saved for each duplex scanning case and $235 saved for each MRA case (not including the $144 cost of post-procedure short-stay unit time required for diagnostic arteriogram.)

Summary—Angiography

Multiple modalities are now available for angiographic imaging of the lower extremity in the diabetic patient. Catheter angiography, now primarily performed using DSA, is considered the gold standard, because it provides the highest potential spatial resolution, including, in particular, spatial detail in the smaller crural and pedal vessels. Catheter angiography carries risks associated with an invasive technique, but also provides the opportunity to combine the diagnostic study with definitive treatment of certain kinds of arterial stenoses. At the same time, noninvasive angiographic imaging techniques have undergone considerable development in recent times and have come to replace conventional DSA for many clinical indications, with DSA being reserved primarily for cases where intervention is required. MRA, CTA, and duplex Doppler ultrasound provide noninvasive alternatives for angiographic imaging and continue to improve their capacity to image subtle disease and small vessels. All types of angiographic imaging are reliant on achieving optimal technique in generating and post-processing of images, in order to attain the highest level of diagnostic accuracy. While the MRI gadolinium-based contrast agents have traditionally been favored over iodinated contrast agents for their low rate of allergic reaction and low incidence of nephrotoxicity, newer concerns regarding the association of nephrogenic systemic fibrosis (NSF) with the use of certain gadolinium contrast agents in patients with renal insufficiency have limited their use in patients with renal failure.

Osteomyelitis Versus Neuroarthropathy

Differentiation between osteomyelitis and neuroarthropathy is often difficult. Certain neuroarthropathic changes resemble osteomyelitis. In order to better understand the similarities and differences, imaging characteristics of neuroarthropathy will be presented here. A more complete discussion of neuro-osteoarthropathic change is provided in another chapter of this book.

Neuroarthropathy

Loss of both pain and proprioceptive sensation is believed to predispose to repetitive trauma, leading to diabetic neuroarthropathy [16]. Though neuroarthropathy is potentially devastating, the reported incidence of neuropathic joints in

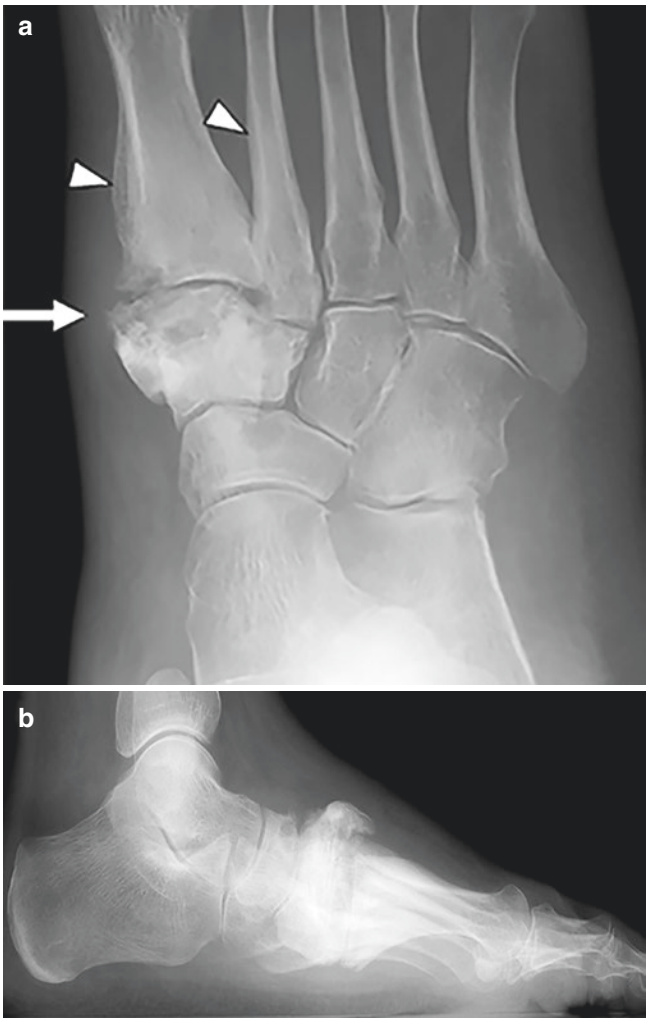

Fig. 5.22 Hypertrophic form of neuroarthropathy. (**a**) AP and (**b**) lateral radiographs show hypertrophic changes in the medial midfoot (arrows), centered about the tarsometatarsal joint. There is bony proliferative change, increased density and non-aggressive periosteal new bone formation (arrowheads) in the 1st and 2nd metatarsal bones, and increased density in the corresponding cuneiforms. In its early phase, this form of neuroarthropathy may be confused with osteoarthritis. Note soft tissue swelling, with effacement of fat planes

the diabetic is surprisingly low, 0.1–7.5% [166]. The joints of the forefoot and midfoot are commonly involved. The distribution of neuroarthropathy in diabetic patients is 24% in the intertarsal region, 30% in the tarsometatarsal region (Fig. 5.22), and 30% in the metatarsophalangeal joints [167]. Abnormalities of the ankle (11%) and interphalangeal (4%) joints are less frequent [167].

Two classic forms of neuroarthropathy, atrophic and hypertrophic, have been described [168]. The atrophic form, representing the acute resorptive or hyperemic phase, is characterized by osseous resorption and osteopenia. This form frequently appears in the forefoot and the metatarsophalangeal joints, leading to partial or complete disappearance of the metatarsal heads and proximal phalanges. Osteolytic changes produce tapering or "pencil-pointing" of phalangeal and metatarsal shafts. Marrow changes in the atrophic or hyperemic form show hypointense T1 and hyperintense STIR and mimic the changes seen in osteomyelitis. The hypertrophic form, representing the healing or reparative phase, is characterized by sclerosis, osteophytosis, and radiographic appearance of extreme degenerative change (Fig. 5.22). In its early phase, the hypertrophic form of neuroarthropathy may be confused with osteoarthritis. Concurrent osseous fragmentation, subluxation, or dislocation predominates in the intertarsal and tarsometatarsal joints. Ruptured ligaments in the mid- and forefoot cause dorso-lateral displacement of the metatarsal bones in relation to the tarsal bones. This classic finding resembles an acute Lisfranc fracture-dislocation (Fig. 5.23). Disruption of the talonavicular and calcaneocuboid joints causes collapse of the longitudinal arch, with subsequent plantar displacement of the talus. These changes produce the classic "rocker bottom" deformity [169]. Recognition of this deformity is important because it creates new pressure points that lead to callus formation and ulceration (Fig. 5.24). Attempts to classify neuropathic joints into the two classic forms may be difficult, as a mixed pattern, composed of both forms, occurs in 40% of neuropathic joints [170]. Traditionally, classification of the natural history of the clinical and radiographic features of neuropathic osteoarthropathy has been based on the Eichenholtz classification, though many updated and alternative systems have also been proposed [166, 171, 172].

Radiography

Radiography is the first-line imaging modality for assessment of suspected neuropathic osteoarthropathy. However, sensitivity for diagnosis of acute neuroarthropathy is relatively low (60%), with specificity of around 80% [166, 173]. Early radiographic findings include soft tissue swelling (which can be minimal), focal demineralization, subchondral fracture (e.g., head of second metatarsal), and periarticular bone resorption [172, 174]. Radiographic findings in chronic Charcot osteoarthropathy are more readily appreciated and include subluxation, dislocation, fractures, bone fragmentation with debris formation, and evidence of "healing" or recrudescence, such as sclerosis at bone edges, osteophyte formation and areas of bone fusion, with resultant overall deformity [172]. Weight-bearing radiographs are helpful in assessing alignment, including pes planus and "rocker bottom" deformity, plantar and dorsal subluxation of metatarsal bases, and Lis franc subluxation and dislocation, and in preoperative planning. Alignment abnormalities demonstrated on standing films have been associated with prediction of ulceration [171, 172, 175, 176].

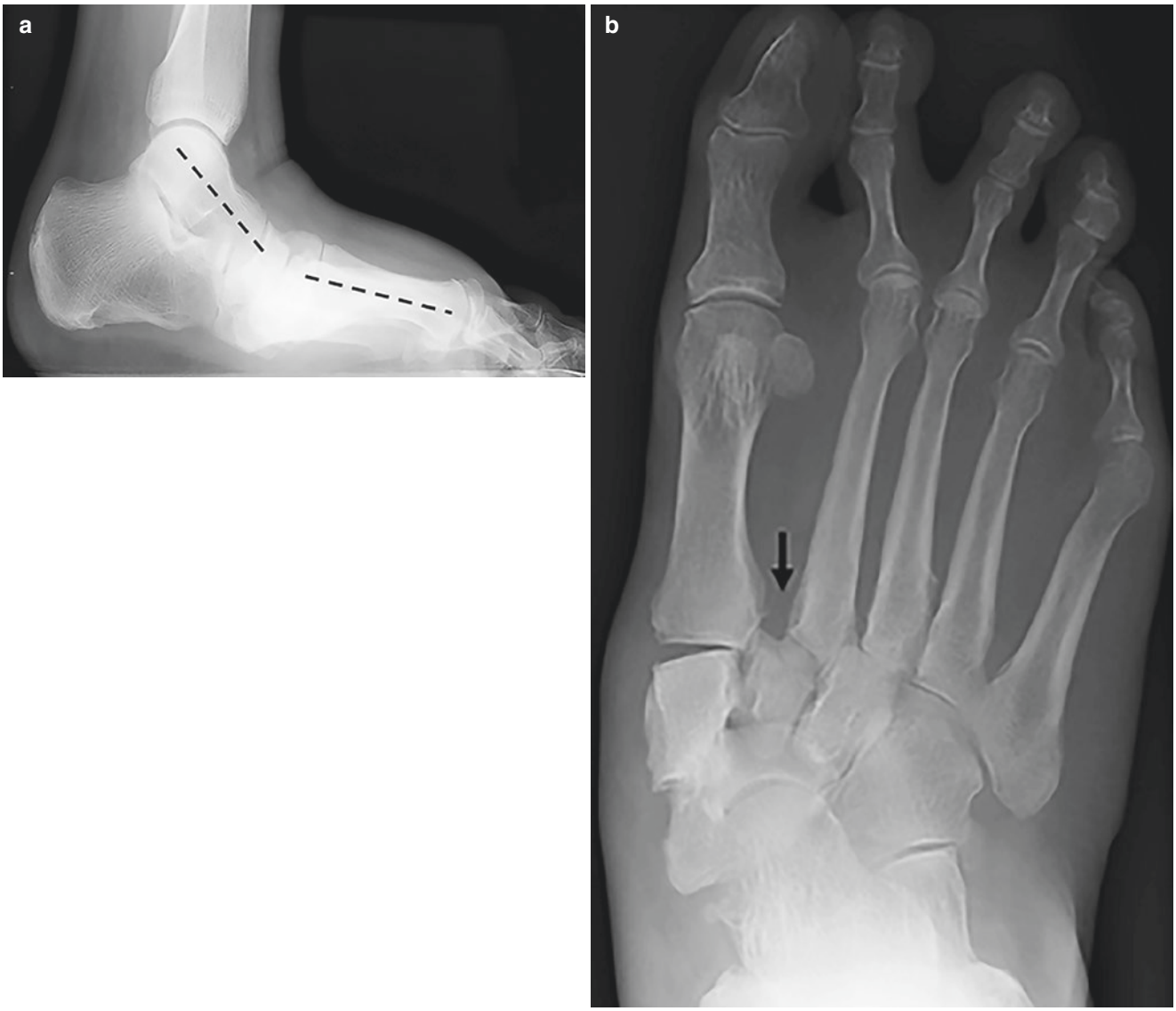

Fig. 5.23 Midfoot deformity related to neuroarthropathy. (**a**) Lateral radiograph demonstrates collapse of the usual longitudinal arch of the foot. Progression can result in extreme "rocker bottom deformity." (**b**) AP view shows Lis-Franc malalignment (arrow) as well as disruption of the navicular-cuneiform articulations

CT

CT has limited sensitivity for detection of bone marrow edema, which limits its utility in the early stage of neuroosteoarthropathy. However, CT can be more sensitive for detection of early fracture and subluxation than radiographs and therefore may help in demonstrating early structural changes of neuroarthropathy, to help in distinguishing it from osteomyelitis, as a cause of soft tissue swelling in diabetic patients who present without soft tissue ulceration [28]. In chronic neuroarthropathy, CT may be useful for preoperative planning [172, 177, 178].

Osteomyelitis Versus Neuroarthropathy

MRI

In the acute phase, MRI findings in neuroarthropathy include soft tissue edema, joint effusion, subchondral marrow edema, disruption of the Lisfranc ligament, and osseous and/or articular disorganization, patchy intraosseous bone marrow on fluid-sensitive sequences, and enhancement of subchondral marrow on post-contrast sequences. In chronic neuroarthropathy, soft tissue edema may persist, however, marrow edema and enhancement decreases.

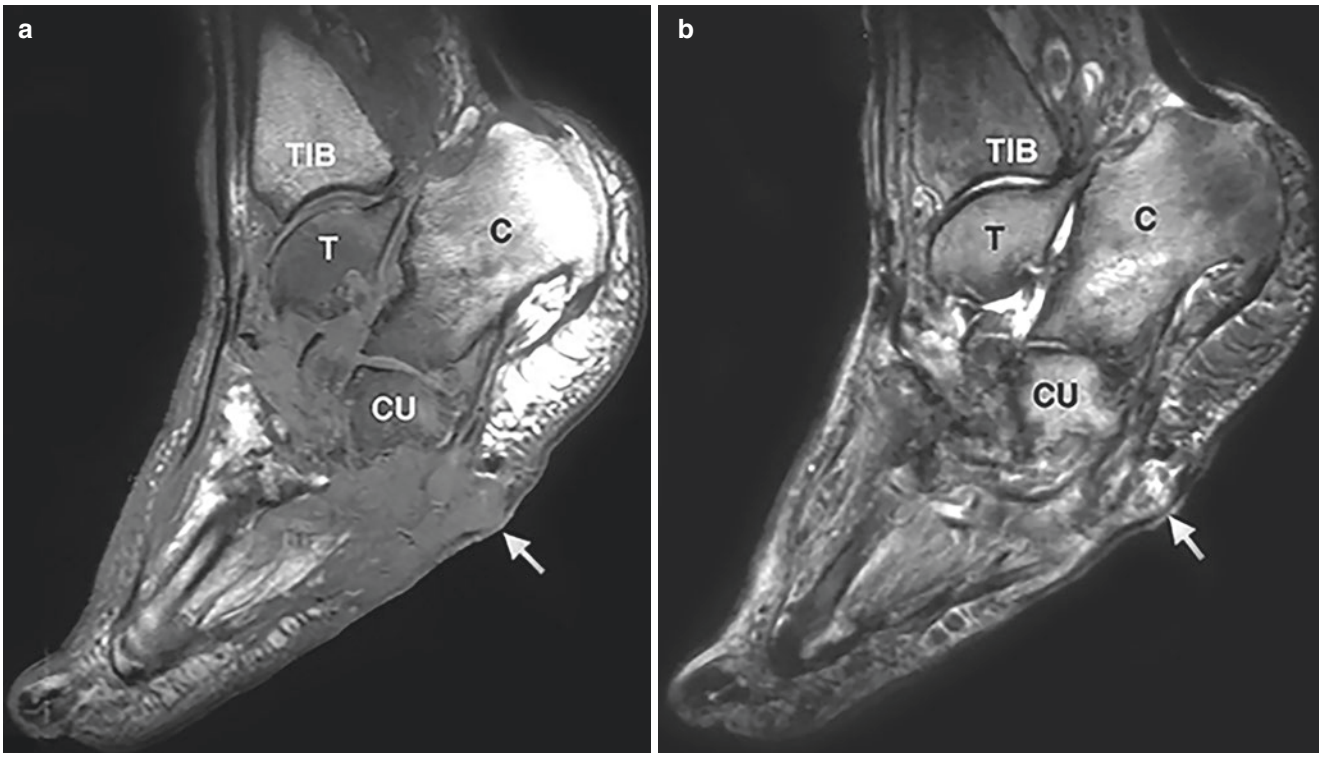

Fig. 5.24 Rocker bottom deformity and ulceration at focus of high plantar pressure on MRI. (**a**) Sagittal T1-weighted and (**b**) STIR images show disruption of the talonavicular joint causing collapse of the longitudinal arch. These changes produce the classic "rocker bottom" deformity. This deformity is important because it creates new pressure points that lead to callus and ulcer formation (arrow). The diffuse marrow edema associated with neuroarthropathy of the tarsal bones mimics osteomyelitis. *T* talus, *CU* cuboid, *C* calcaneus, *TIB* tibia

Table 5.6 Osteomyelitis vs. neuroarthropathy

	Favors osteomyelitis	Favors neuroarthropathy
Radiography		
Location	Forefoot, metatarsal heads and toes	Mid foot
Cortical destruction	Discrete cortical lesion	Absent
Proximity to soft tissue ulcer	Beneath or close to the ulcer or soft tissue infection	Some distance from soft tissue infection or ulcer
MRI		
Signal characteristics of the abnormal marrow	Hyperintense STIR or T2 marrow signal (this signal pattern is nonspecific and overlaps the hyperemic form of neuroarthropathy and acute fracture)	Hypointense marrow signal on all T1, T2 and STIR sequences (this signal pattern corresponds to the hypertrophic form of neuroarthropathy)
Cysts	Not common in osteomyelitis	Well-marginated cyst-like lesions, hypointense on T1 and hyperintense on T2
Other	Gadolinium contrast outlining fistulous tract between ulcer and bone with abnormal marrow	
Osteomyelitis superimposed on neuroarthropathy	– "Ghost sign"—Cortical margins that are indistinct on T1W images, but distinct on T2W and contrast-enhanced images – Disappearance of previously seen subchondral cysts and loose bodies	

Subchondral cysts (rounded low T1/high T2 foci) and linear areas of low T1 signal as well as subluxation, dislocation, and bone fragmentation and hypertrophy are seen [179]. Other than the characteristic findings of diffuse dark marrow signal on T1-, STIR and T2-weighted MR images associated with hypertrophic neuroarthropathy (versus high T2 and STIR signal seen in osteomyelitis), there is no easy method of distinguishing between osteomyelitis and neuroarthropathy. Secondary findings such as involvement of the midfoot and multiple joints, absence of cortical destruction, presence of small subchondral cyst-like lesions, and distance between soft tissue infection and bone changes favor a diagnosis of neuroarthropathy (Table 5.6). In contrast, osteomyelitis favors the toes or metatarsal heads, cal-

caneus, and malleoli, and is associated with focal cortical lesions and close proximity to the ulcer. Marrow changes in osteomyelitis tend to occur on one side of the joint (unless associated with septic arthritis), while neuroarthopathic changes tend to occur on both sides of a joint [179]. When assessing for potential superinfection of a neuroarthropathic joint, signs include total effacement of the adjacent soft tissue fat signal, larger than expected fluid collections in soft tissues, and interval disappearance of the subchondral cysts and/or intra-articular loose bodies. When cortical margins are indistinct on T1-weighted images, but appear distinct on T2-weighted or contrast-enhanced images ("ghost sign"), that is also suggestive of superimposed osteomyelitis [106]. Ultimately, differential diagnosis may require aspiration of joint fluid or percutaneous biopsy, though care should be taken to avoid introducing infection into noninfected bone [172, 179].

Radionuclide Studies

Technetium 99m methylene diphosphonate bone scan will show increased activity in areas of increased bone turnover, but this finding is nonspecific and can be seen with trauma, postsurgical change and infection. As a result, changes of osteoarthropathy can result in increased activity on bone scan and can cause a false-positive scan for osteomyelitis.

Keidar et al. found that ^{18}FDG uptake on PET scans was increased in both infection and osteoarthropathy [48]. However, several recent studies have suggested a potential future role for FDG-PET in distinguishing neuroarthropathy from osteomyelitis [55, 180, 181].

Imaging Algorithm: Approach to Diagnosis of Pedal Osteomyelitis in the Diabetic Patient

A suggested algorithm for imaging pedal osteomyelitis in the diabetic patient is presented in Fig. 5.25.

Soft Tissue Ulceration Exposing Bone

When a soft tissue ulcer exposes bone, there is a relatively high positive predictive value for osteomyelitis [1, 21]. Radiography is appropriate to provide a baseline and to document bone complications. MRI may be useful for preoperative planning, as it provides detailed anatomic landmarks for bone and soft tissue pathology and has a high negative predictive value for osteomyelitis and soft tissue infection, thus demarcating normal bone and soft tissue.

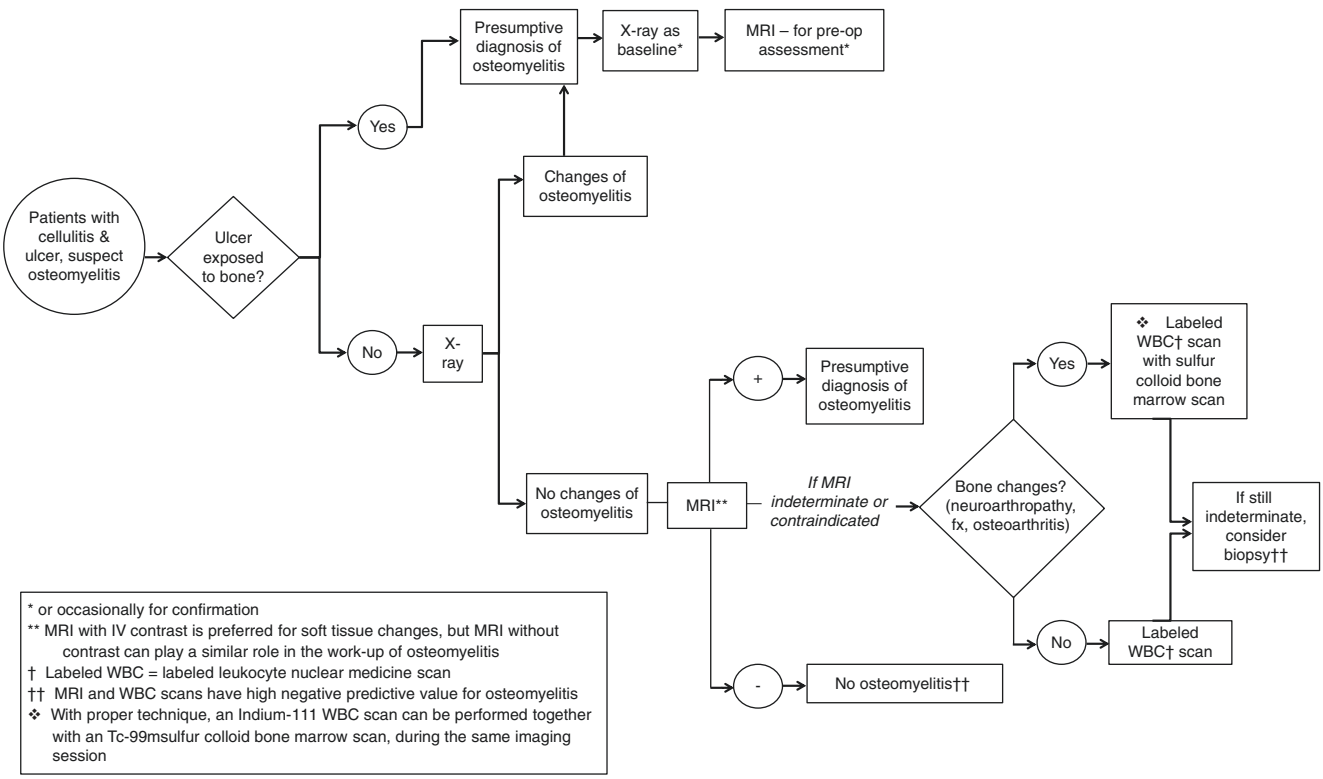

Fig. 5.25 Suggested approach to diagnosis of osteomyelitis in diabetic foot infection

Soft Tissue Inflammation (Ulcers and/or Cellulitis) with No Exposed Bone

Radiographic findings are used to further separate the patients into two groups: (1) those who have obvious changes of osteomyelitis on their foot radiographs, yielding a presumptive diagnosis of osteomyelitis, and (2) those whose foot radiographs appear normal.

If radiographs show characteristic changes of osteomyelitis, an MRI may be performed for preoperative planning, in order to map the extent of the abnormalities and localize any devitalized areas.

If the radiographs show normal bone and the clinical suspicion for osteomyelitis is high, then an MRI will help to demonstrate the presence and distribution of bone and soft tissue infection [179]. Although the use of intravenous (IV) gadolinium contrast is generally preferred because it can help delineate fistulous communication between an ulcer and areas of bony abnormality, and also will outline soft tissue abscesses, if IV contrast is contraindicated, e.g., due to renal insufficiency, then MRI performed without IV contrast can still be very useful in demonstrating areas of bone and soft tissue abnormality. An MRI with classic findings of osteomyelitis provides a presumptive diagnosis of osteomyelitis. A normal MRI has a high negative predictive value and effectively excludes osteomyelitis. Occasionally, an MRI may be indeterminate, particularly in cases when distinction between osteomyelitis and neuroarthropathy is difficult. While certain MR imaging features favor osteomyelitis versus changes of osteoarthropathy, in some cases the distinction between osteomyelitis and osteoarthropathy may be difficult [179]. In those cases, additional workup is required, as detailed below.

If MRI is contraindicated or not available, then a labeled leukocyte (white blood cell) scan can serve as an effective alternative [28]. When changes of osteoarthropathy are present, then a technetium 99-m sulfur colloid bone marrow scan, supplementary to the labeled leukocyte scan, may be helpful [43]. Although nuclear medicine bone scan studies have long been the mainstay for imaging of osteomyelitis, they are no longer considered a first-line nuclear medicine for evaluation of osteomyelitis [27].

Although CT has a limited role in the imaging workup of osteomyelitis, if radiographs are normal and suspicion for osteomyelitis is low, then CT may help to demonstrate early changes of osteoarthropathy [28].

Equivocal MRI

If the MRI is equivocal for osteomyelitis, then further imaging workup could include a labeled leukocyte scan and, if there are changes of osteoarthropathy, a comparative technetium 99m sulfur colloid bone marrow scan [28, 182]. Labeled leukocytes can accumulate in an uninfected neuropathic foot [43] and a correlative technetium-99m sulfur colloid bone scan helps to differentiate labeled leukocyte activity due to bone marrow displacement versus osteomyelitis. A study is positive for osteomyelitis when uptake is greater in either intensity or distribution on the labeled leukocyte scan, compared with the bone marrow scan [43]. In practice, an indium-111-labeled leukocyte (WBC) scan and a technetium-99m sulfur colloid bone marrow scan can both be performed in a single "sitting," rather than as sequential studies. In the complex anatomy of the mid- and hindfoot, SPECT/CT also may be a useful adjunct to labeled leukocyte scanning, in order to help determine whether increased activity is located in the soft tissues or in the bone [29, 30].

Conclusion

Imaging plays an important role in the assessment of the diabetic patient with foot problems. Nuclear medicine and MRI techniques detect osteomyelitis, characterize various soft tissue abnormalities, and depict the extent of bone involvement. Digital subtraction angiography and noninvasive angiographic studies can be used in complementary fashion to evaluate lower extremity arterial anatomy and pathology. Nevertheless, distinguishing osteomyelitis from coincident neuropathic change remains a challenge. Only with an understanding of the specific strengths and weaknesses of each modality, as they apply to the particular clinical problem in question, can this wide variety of imaging studies be utilized in an effective and efficient manner.

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Principles of Care in the Diabetic Surgical Patient

6

Natasha Khazai and Osama Hamdy

Abstract

Currently, around 29 million or 11% of US adults have diabetes and 86 million are thought to have prediabetes. The healthcare spending on diabetes is soaring with an estimate of $250 billion, of which 43% are spent in hospital care to treat diabetes and its complications. Patients with diabetes are frequently admitted to the hospital for surgical interventions. Common surgical reasons in relation to diabetes include diabetic foot problems, vascular surgeries, coronary artery bypass, kidney transplant, and eye surgeries. Meanwhile, patients with diabetes are frequently admitted for any other surgical interventions unrelated to diabetes. During admission, diabetes management may vary based on the location of patient whether in surgical intensive care units or in regular wards. It also varies based on nutrition whether it is regular oral feeding, enteral tube feeding, parental feeding, or just clear intravenous fluids. Good diabetes control shortens length of hospital stay, reduces complications, and reduces hospital mortality and hospital 30- and 90-day readmission rate. While oral medications and/or insulin are commonly used to treat diabetes in outpatient setting, only insulin is recommended for treating diabetes during surgical admission. Insulin method of administration, insulin type, and dose vary significantly between patients. Use of steroids may complicate insulin regimen. Patients on insulin infusion pump also require specific consideration. The major risk of insulin use is hypoglycemia, which is infrequently severe. This chapter comprehensively covers the principals of diabetes management during hospital admission.

Rationale

In hospitalized patients, both hyperglycemia and hypoglycemia have been associated with poor outcomes. During the inpatient period, hyperglycemia has been associated with increased risk of infection [1, 2], cardiovascular events [3–5], and mortality [6, 7]. It is also associated with longer length of hospital stay [3, 8, 9]. Hypoglycemia has been also associated with an increased risk of mortality [10]. Therefore, current evidence supports avoidance of both conditions among hospitalized patients whether they are admitted to critical care units or non-critical care units [5, 9, 11].

Glucose Targets in Non-critically Ill Patients

Unfortunately, due to limited number of trials, optimal blood glucose (BG) targets are still debated [12]. Glucose targets recommended by the consensus guidelines issued jointly by the American Diabetes Association (ADA) and the American Association of Clinical Endocrinologists (AACE) [9] and separately by the Endocrine Society [5] are shown in Table 6.1. Higher BG targets can be set for those

Table 6.1 Blood glucose targets in inpatient setting for non-critically ill patients according to joint consensus of the American Diabetes Association (ADA) and the American Association of Clinical Endocrinologists (AACE) [9]

Random or bedtime (mg/dL)	Fasting and premeal (mg/dL)	Hypoglycemia (mg/dL)
<180	100–140	<70

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who are prone to hypoglycemia, have severe comorbidities, or are terminally ill [11]. However, even then, it is recommended that BG be kept below 200 mg/dL in order to avoid symptomatic hyperglycemia [11]. The basal-bolus insulin doses should be reduced if BG falls below 100 mg/dL, unless the patient is clinically stable and has tight control before admission [11].

Diabetes Management for Non-critically Ill Hospitalized Patients

In all patients with diabetes, hemoglobin HbA1c (A1C) should be checked upon admission unless patient has a value within the past 3 months [5, 9, 11]. For patients without diabetes, who have random blood glucose level exceeding 140 mg/dL whether in the emergency room or during hospitalization, A1C should be checked. If A1C is ≥6.5%, it strongly suggests that diabetes preceded hospitalization [11]. If random blood glucose is >140 mg/dL but A1C is ≤6.4, diabetes diagnosis can't be totally excluded and oral glucose tolerance test should be ordered after discharge.

Involvement of a diabetes educator early in the course of admission may help newly diagnosed patients to learn few essential skills that may help them upon discharge like glucose monitoring, prevention and management of hypoglycemia and proper intake of oral antihyperglycemic medications [9, 11]. The same is true for patients starting insulin for the first time, where involvement of a diabetes educator not only ensures they receive instructions and have hands-on practice on proper insulin injection technique, but also may identify barriers to self-management such as poor patient dexterity that precludes insulin self-administration. This may allow for early involvement and teaching of the patient's caregiver, or if needed a change in the patient's diabetes discharge plan to a one that either patient or his/her caregiver is able to execute. Early identification and management of these issues ensure safe and timely discharge [13].

Medical Nutrition Therapy

Medical nutrition therapy is important for both outpatient and inpatient diabetes management. All patients with diabetes should be on a balanced, hypocaloric and carbohydrate consistent diet. If enteral nutrition is used, a diabetes-specific formula is preferred over standard formula. Carbohydrate consistency helps in proper matching of prandial insulin with carbohydrate content of the meals [8]. Carbohydrate should be from whole grains, vegetables, fruits, and low fat dairy with restricted amounts of added sugar and sucrose-containing foods [14].

Oral Antihyperglycemic Medications and Glucagon-Like Peptide-1 Receptor Agonists (GLP1-RA)

The most recent guidelines from ADA, AACE, and Endocrine Society recommend against the inpatient use of oral antihyperglycemic medications or GLP-1-RA, due to lack of efficacy studies and because of safety issues [5, 9, 11]. Metformin use in hospitalized patients may potentially lead to lactic acidosis, in the event of continued use during renal insufficiency, sepsis, hypotension, or a hypoxic state such as heart failure. Sulfonylureas are associated with increased risk of hypoglycemia, especially upon unpredicted discontinuation of a patient's diet oral feeding (NPO). In case of declining renal function, hypoglycemia due to sulfonylureas may become severe and protracted. Few studies investigating the use of GLP-1RA among hospitalized patients with type 2 diabetes showed non-inferior glycemic control and hypoglycemic event rates, when compared with basal-bolus insulin [15]. However, these therapies often need additional basal insulin therapy to maintain optimal glycemic control. Furthermore, GLP-1RA therapies are associated with early gastrointestinal side effects such as nausea and vomiting in up to 66% of patients. These side events are specifically undesirable in already anorexic patients or in patients who are sedated as they put them at higher risk for aspiration pneumonia [16]. Hospitalized patients may continue their oral medications and/or GLP-1RA only if they meet all the criteria listed in Box 6.1, while taking into consideration all the precautions listed in Box 6.2. There are some early indications that well-tolerated non-hypoglycemic agents such as a DPP-4 inhibitor may be used in an inpatient setting. All other patients should be treated with insulin. It needs to be noted that if oral agents are held, treating physician should have a plan to resume them 1–2 days before discharge to ensure their efficacy and safety [11].

Box 6.1: Criteria for Continuing Oral Antihyperglycemic Medications and GLP-1 RA During Hospital Admission

- Low risk, stable patient
- Hemoglobin A1C < 8%
- Eating >50% of diet
- Expected discharge within 24–48 h
- No plans for contrast studies
- No acute renal failure
- No steroid therapy
- No infection

Box 6.2: Cautions for Use of Oral Antihyperglycemic Medications and GLP-1 RA During Hospital Admission

Metformin

- Discontinue if Cr is >1.4 mg/dL and/or eGFR is <60 mL/min per 1.73 m^2
- Hold for 48 h after IV contrast study and check renal function daily for 2 days [17]
- Discontinue in hypoxic states (CHF, COPD exacerbation, sepsis)
- Discontinue if patient has liver disease

Sulfonylurea

- Discontinue if patient has renal acute or chronic insufficiency
- Discontinue if patient is made NPO

Pioglitazone

- Discontinue if patient has congestive heart failure or lower extremity edema

GLP1-RA

- Discontinue if patient develops pancreatitis, nausea, or vomiting

Insulin

Patients with established or newly diagnosed diabetes that have a good nutrition plan should be started on long-acting basal insulin plus rapid-acting bolus (nutritional) insulin for meals plus a corrective dose of same rapid-acting insulin while in the hospital [5, 9]. Those who have poor nutritional intake or are NPO should receive basal insulin along with corrective doses of short-acting insulin [9, 15]. Once they resume nutritional intake, a safe step can be administering nutritional (bolus) insulin right after the patient eats in order to allow for better matching of insulin with actual carbohydrate intake [11]. Patients who are well trained and used carbohydrate counting should have the option to continue using the same outpatient insulin-to-carbohydrate ratio to calculate their nutritional insulin needs for each meal. The use of a "sliding scale" (corrective) insulin alone without basal and nutritional insulin is strongly discouraged (except in select cases, see Table 6.2), with strong evidence showing its inferior performance compared to a basal-nutritional-corrective regimen [18]. The basal, bolus, and corrective insulin dose for each patient depends on a number of factors. These factors include presence or absence of diabetes, type of diabetes, admission A1C level, and how diabetes was managed prior to admission. It is expected that patients with type 2 diabetes who are well controlled on oral medications as outpatient will need a smaller total daily dose (TDD) of insulin compared to patients with type 2 diabetes who are poorly controlled on insulin as outpatient. Suggested guidelines for calculating TDD of insulin are summarized in Table 6.2 [5, 9, 19, 20]. It needs to be stressed that similar to outpatient setting inpatient diabetes management also needs individualization for each patient and that the suggested calculations should only serve as starting points. Of note, for patients who are inadequately controlled (A1C > 10%) on oral antihyperglycemic medications with or without GLP-1 RA as outpatients, basal insulin will likely be added to their outpatient diabetes regimen upon discharge. Early involvement of a diabetes educator for insulin teaching is strongly advised for those patients. Patients, who were well controlled on basal and nutritional insulin as outpatients, can be continued on their home dose of basal insulin. It is recommended to reduce home doses of their nutritional insulin by 25–50% initially to avoid hypoglycemia in case carbohydrate content in their hospital meals is significantly less than their diet at home. On the other hand, patients who were adherent to their insulin regimen at home but were poorly controlled (A1C > 10%) should have their TDD calculated based on Table 6.2. If calculated TDD is lower than what they were using at home, they should be started on their outpatient regimen with daily up-titration of their TDD based on BG response in the hospital. Frequently, poor outpatient control among those patients is related to poor dietary adherence. This poor dietary adherence is mostly eliminated in the hospital with institution of a calorie-restricted, carbohydrate-consistent diet. Calculation of basal, nutritional, and corrective insulin doses based on TDD are outlined in Table 6.3. It was shown that patients with renal insufficiency (eGFR < 60 mL/min per 1.73 m^2) who started on glargine or detemir insulin using a lower

Table 6.2 Calculation of total daily dose (TDD) of insulin

Patient and glycemic profile	TDD units/kg of body weight
Oral agents or lifestyle therapy as outpatient, A1C < 7% Newly diagnosed patients, A1C < 7%	Consider corrective insulin only If BG is consistently >140 mg/dL, add basal insulin (0.1 unit/kg body weight)
Oral agents as outpatient, A1C 7–7.9% Any treatment and age ≥70 years and/or eGFR < 60 mL/min per 1.73 m^2 [21]	0.2–0.3 unit/kg body weight
Any DM with blood glucose 140–200 mg/dL or admission A1C < 10%	0.4 unit/kg body weight
Any DM with blood glucose 200–400 mg/dL or admission A1C ≥10%	0.5 unit/kg body weight

Table 6.3 Basal-bolus (nutritional) and corrective insulin dose calculation

Basal insulin	Starting dose = TDD[a] × 0.5 – Glargine insulin: One dose at bedtime or – Detemir insulin: One dose at bedtime (Type 2) or split to 2 equal doses AM and bedtime (Type 1) or – NPH insulin: 2/3 AM and 1/3 bedtime – Premixed insulin 70/30, 75/25, or 50/50 is not generally recommended in the hospital unless patient needs to be discharged on this regimen **Note 1:** Glargine and detemir are preferred over NPH in the hospital setting (lower risk of hypoglycemia as NPH peak may seriously decrease blood glucose when patients are fasting for a procedure or any other reason) **Note 2:** Use NPH if short hospital stay is anticipated and patient is unable to afford/switch to glargine/detemir as outpatient. NPH is also preferred in patients on oral steroid therapy
Nutritional (bolus) insulin	Starting dose = TDD[a] × 0.5 divided equally before each meal – Lispro, aspart, and glulisine are preferred over regular insulin for hospitalized patients (less risk of hypoglycemia) **Note 3**: Inject 50% or less of calculated nutritional insulin if patient has reduced intake **Note 4**: Hold nutritional insulin if patient is not able to eat
Corrective insulin	**CF** (correction factor) = 1700 ÷ TDD[a] This means that 1 unit of insulin will lower BG by **CF** mg/dL, therefore: Corrective insulin dose or STAT dose = (current BG - 100) ÷ **CF** – Build the scale by increasing insulin dose by 1 unit for every CF – Give nutritional and correction doses as 1 injection with meals **Example:** A 80 kg patient with type 2 diabetes and A1C of 11% needs: **TDD** = 60 kg × 0.5 = 40 units **Basal insulin** = TDD × 50% =20 units of glargine or detemir insulin qhs **Nutritional insulin** = 20 ÷ 3 = ~7 units rapid-acting insulin with each meal **Correction factor** = 1700 ÷ 40 = 42 mg/dL *This means that 1 unit of insulin is expected to lower BG by ~40 mg/dL* corrective insulin dose = (BS-100)/CF A scale can be made as follows: Premeal corrective insulin scale (BG goal < 140 mg/dL) Scale: 140–180 mg/dL = 1 unit 181–220 mg/dL = 2 units 221–260 mg/dL = 3 units etc. Bedtime corrective insulin scale (BG goal < 180 mg/dL) Scale: 141–180 mg/dL = 0 unit 181–220 mg/dL = 1 unit 221–260 mg/dL = 2 units etc.

[a]TDD is calculated using instructions in Table 6.2

multiplier of 0.2 (instead of 0.5) multiplied by patient's body weight had reduced incidence of hypoglycemia by around 50% [21].

Adjusting Basal and Bolus (Nutritional) Insulin

When adjusting insulin doses, one has to take into account patient's clinical status, concomitant medications (see Glucocorticoid section), blood glucose values, individualized glucose targets, and nutritional status (see Enteral and Parenteral section) among other factors [9]. Insulin adjustment, therefore, is a highly individualized process. Some guidelines that are commonly used for adjusting basal and nutritional insulin, along with examples to help highlight these guidelines, are shown in Table 6.4.

Computerized Provider Insulin Order Entry

Computerized provider order entry (CPOE) for insulin has shown to significantly improve percent times of patient's BG is within target range and lower mean BG without an increase in hypoglycemic events [22, 23]. Institution of a CPOE is a core requirement of Health Information Technology and Clinical health Act (HITECH) and is also recommended by the Institute of Medicine [11]. Routine structured order sets for basal, nutritional, and corrective insulin should be made part of CPOE. Ordering individualized corrective insulin scales, using the patient's calculated correction factor, can be made possible in such computerized order sets.

Glucose Monitoring

Blood glucose should be checked before meals and at bedtime. For patients who are NPO, frequency may be increased to every 4 h while awake. For patients who are at risk of hypoglycemia, a 3 am blood glucose check is recommended [11]. Glucometers that connect wirelessly to the hospital's electronic health record system can greatly facilitate and expedite needed changes in the patient's insulin orders and prevent recurrent hypo- or hyperglycemia. Continuous glucose monitoring (CGM) promises to reduce the incidence of

Table 6.4 General guidelines for adjusting basal and nutritional insulin

Fasting BG > 140 mg/dL	– Increase bedtime long-acting insulin. If NPH or detemir are used q12 hours, increase HS dose: • 10% if FBG is 140–199 mg/dL • 20% if FBG is 200–299 mg/dL • 30% if FBG is 300–399 mg/dL Example: FBG: 190 mg/dL, prelunch: 135 mg/dL, predinner: 120 mg/dL, bedtime: 140 mg/dL. Patient is on 40 units of glargine insulin at bedtime → Increase basal glargine insulin by 10% from 40 units to 44 units
Premeal BG > 140 mg/dL or bedtime BG > 180 mg/dL and Fasting BG < 140 mg/dL	– Increase nutritional rapid-acting insulin: • 10% if BG is 140–199 mg/dL • 20% if BG is 200–299 mg/dL • 30% if BG is 300–399 mg/dL Example: FBG: 120 mg/dL, prelunch: 200 mg/dL, predinner: 230 mg/dL, bedtime: 280 mg/dL. Total nutritional insulin is 20 units → Increase nutritional insulin by 20% from 20 units to 24 units
Fasting and premeal BG > 140 mg/dL and Bedtime BG > 180 mg/dL	→ Increase both prandial and basal insulin as shown above

severe hypoglycemia, but more research is needed to establish its accuracy and reliability in hospital setting [11]. Therefore, CGM is not currently recommended for routine hospital use except for patients who are already using them as outpatients and wish to cautiously continue using them in hospital.

Corticosteroids

Glucocorticoid-induced hyperglycemia, defined as blood glucose levels >180 mg/dL after initiation of glucocorticoids, has been reported in 32% [24] to 52% [25] of inpatients. Of these, 18% [24] to 25% [25] were diagnosed with diabetes. Hyperglycemia in these patients has been shown to be associated with an increased risk of mortality [26], infections [27], and length of stay [28]. Despite the importance of controlling hyperglycemia in these patients, not enough head-to-head randomized controlled trials exist to recommend a specific type or a starting dose of insulin for a certain type of steroid. Since hyperglycemia in response to morning oral steroid is predominately seen from noon time till evening, an additional single dose of NPH given in the morning should be most effective [11] compared to adjusting basal-bolus (even when the noon and pre-supper bolus doses are increased) [29]. In the only single randomized controlled trial done to date [19], when NPH was added onto the patient's basal-bolus-corrective regimen there was a trend toward improved glycemic control without increased risk of hypoglycemia, compared to adjusting patient's basal-bolus-corrective insulin alone. This seems intuitive because NPH insulin peaks 4–10 h post injection, around the same time prednisone exerts its hyperglycemic effects (4–8 h). In addition, NPH has a duration of action of approximately 12–18 h similar to the duration of hyperglycemic effects of prednisone [30]. A slightly simplified version of an insulin protocol for patients on glucocorticoids by Grommesh et al. [19] is shown in Table 6.5. In this protocol, starting dose of NPH depends on the patient's prednisone dose and absence or presence of diabetes. It needs to be emphasized that the NPH dose has to be added on to the patient's existing basal-bolus and corrective dose. Instead of using a fixed NPH dose, NPH dose can be calculated as 0.27 units/kg [31].

Table 6.5 NPH insulin dose administered at the time of glucocorticoid administration[a]

	Prednisone <40 mg/day as single morning dose	Prednisone ≥40 mg/day as single morning dose
Hyperglycemia but no history of diabetes	5 units	10 units
Established diabetes	10 units	20 units
– Increase NPH by 25% if BG > 180 mg/dL, and increase by 50% if BG > 300 mg/dL – Taper NPH by same percentage as prednisone is tapered – NPH can be stopped when prednisone dose is reduced to <10 mg/day		

[a]It is recommended that a mechanism be implemented in the CPOE that links the prednisone order to the NPH order, such that it is given at the same time, and signals a need for change or hold in NPH if glucocorticoids are changed or held

Multiple daily doses of glucocorticoids such as hydrocortisone and methylprednisolone, and longer-acting glucocorticoids such as dexamethasone, may be better controlled with longer-acting insulin like glargine and detemir insulin [11]. A retrospective study by Gosmanov et al. [32], assessing glycemic control of patients with diabetes and hematologic malignancies who were receiving dexamethasone, found that a daily-adjusted basal-bolus regimen achieved lower average blood glucose levels compared to a fixed sliding scale insulin. For patients starting dexamethasone therapy, a TDD is somewhere between 0.66 and 1.2 units/kg.

In those patients who remain uncontrolled with BG levels >400 mg/dL on a subcutaneous regimen, an intravenous insulin infusion should be considered.

Table 6.6 Diabetes management for patients on enteral and parenteral nutrition

Continuous enteral nutrition	**Method 1** Initial TDD = 0.3–0.6 unit/kg body weight • ½ TDD as basal: Glargine insulin q24hr • ½ TDD as "prandial": NPH q8–12[a] or regular q6hr • Corrective insulin: Regular q6hr[b] • BG checked q6hr • "Prandial" insulin to be held if parenteral nutrition stopped • "Prandial" insulin is adjusted by adding 80% of previous day's correctional insulin to current prandial dose[c] **Method 2** TDD given as just basal: 2 doses of NPH or detemir 1 dose of glargine[d] Corrective insulin: Regular insulin q6hr[b] • Basal insulin is adjusted by adding 50% of previous day's correctional insulin to current basal dose[c] • If parenteral nutrition stopped, give only 0.3 unit/kg basal insulin along with corrective insulin
Cyclic enteral nutrition	• Continue existing basal, bolus, and corrective insulin • In addition, give extra insulin dose for cyclic EN = 60% of (0.3–0.6) unit/kg body weight • Given as NPH/regular, or premixed 70/30 (or 75/25) at the time of TF initiation • Titrate based on midnight, 3 am and 6 am BG
Bolus enteral nutrition	• Continue existing basal, bolus, and corrective insulin • Add additional rapid-acting insulin for each bolus feeding • BG checked before meals and bedtime
Total parenteral nutrition (TPN)	• Continue existing basal, bolus, and corrective insulin • Add insulin to TPN bag (0.1unit per 1 g of dextrose) • Titrate 0.05 units per 1 g of dextrose daily if BG >150 mg/dL • BG checked q6hr
Interruption of enteral feedings	• Adjust insulin appropriately with planned withholding of enteral nutrition • Standing orders for "prandial" insulin to be held and MD notified if enteral feeds are to be stopped at any point. • In the event of unexpected and abrupt interruption of enteral feedings exceeding 2 h start D10W intravenously at the same rate as the enteral feedings were given to prevent hypoglycemia and dehydration

[a]This decreases injection frequency while still remaining a good option for lowering the risk of hypoglycemia if enteral nutrition is abruptly stopped

[b]Please see Table 6.3 for how to calculate this dose

[c]If previous day correctional insulin is added to basal insulin, a higher risk of hypoglycemia ensues upon abrupt or planned discontinuation of enteral nutrition. In the former case, providers might overestimate the true basal insulin needs

[d]Higher risk of hypoglycemia with this regimen if enteral nutrition is abruptly stopped

Enteral and Parenteral Nutrition

It is recommended that patients be started on nutritional support within 24–48 h if they are critically ill, or after 7–14 days if they are not critically ill [33] but unable to meet >60% of nutritional needs by mouth. The recommended glycemic goal is less than 180 mg/dL (Table 6.1), however observational studies suggest that a lower BG target of <150 mg/dL improves clinical outcomes for those receiving nutritional support without increasing hypoglycemia risk [34].

Enteral nutrition: Multiple randomized controlled trials support the use of lower carbohydrate content (diabetes-specific) enteral formulas due to their association with reduced hyperglycemia [35–39]. In standard enteral formulas, 55–60% of the calories are provided by carbohydrates, whereas diabetic specific formulas reduce carbohydrates contribution a maximum of 40% of total caloric content. This is made possible by substituting some of the carbohydrate content with monounsaturated fatty acids and dietary fiber [40, 41]. A variety of insulin regimens are used to manage hyperglycemia for patients on parenteral nutrition. Superiority of one regimen over the other remains to be established. To date there have been several retrospective [42] and only one randomized controlled trial (RCT) examining this question [40]. This RCT [43] showed that one dose of glargine insulin with corrective regular insulin performed just as well as NPH twice daily with corrective regular insulin, with no difference in target blood glucose achieved or hypoglycemic events [44] (see Method 1, Table 6.6 [45]).

Total parenteral nutrition: Administering insulin directly into the TPN solution is associated with lower hypoglycemia risk in the event of abrupt TPN discontinuation. For patients with diabetes, start with 0.1 unit or regular insulin per 1 g of dextrose with daily titration by 0.05 units per 1 g dextrose if blood glucose remains above 150 mg/dL [46]. For patients without diabetes the unit per dextrose grams ratio has been shown to be lower at 0.1 unit per 2 g of dextrose [47].

Insulin Pump Management in the Hospital

Approximately 30–40% of type 1 diabetes patients and an increasing number of insulin requiring type 2 patients are using insulin pumps [48]. Majority of insulin pump users are well trained on diabetes self-management and frequently get frustrated when they are asked to stop using their insulin pump during hospitalization. Adding to this frustration is the non-intentional delay in administering their bolus or corrective insulin by hospital staff. It is recommended that insulin pump users be allowed to self-manage their diabetes during hospital provided if they are able to demonstrate adequate skill and ability to manage their pumps and are able to procure their pump supplies [11, 49]. An "insulin pump agreement" helps outline expectations that patient needs to collaborate and communicate with hospital team by reporting blood glucose and basal levels, and any boluses given for meals or correction. These values need to be documented on a special "Insulin pump record sheet." A hospital-wide insulin pump policy can help in smoothing transition to SC insulin in the event of pump failure, or change in the patient's cognition that prevents continued self-management. This policy should delineate responsibilities for hospital team members and ensure ultimate patients safety. Involvement of a diabetologist, either on site or by phone, is highly recommended to assist in recommending changes in basal, bolus, or corrective settings while patient is in the hospital [48, 50].

In the event of surgery: Patients who are on an insulin pump can continue their usual basal rate during minor surgeries and major noncardiac surgeries lasting less than 6 h, providing that their ongoing basal rate is not causing hypoglycemia. If there is any concern about possible fasting hypoglycemia, a temporary basal rate should be set at 80% of the patient's usual basal rate. The infusion set should be changed 24 h before surgery and insertion site should be selected away from the surgical site. The chosen site can be anywhere on the upper outer thighs, upper arms, or abdomen 2 inches away from the umbilicus. Blood glucose should be checked every hour during surgery. Transition to insulin infusion should be considered if blood glucose exceeds and remains above 180 mg/dL.

Critical illness: Patients on insulin pumps need to be transitioned to an intravenous insulin infusion in critical illness [48].

Perioperative Diabetes Management

Patients with diabetes should be given preference for early morning surgery. Doing so may decrease risk of hyperglycemia and hypoglycemia resulting from disruption in typical medication and food schedules. On the day prior to surgery, patients with diabetes should continue their usual hospital ordered calorie-restricted, carbohydrate-consistent diabetic diet along with their ordered insulin and/or oral antihyperglycemic medications. Changes that need to be made to the patient's diabetes medication regimen are listed in Table 6.7. Patient's healthcare team needs to ensure that patient is not sent to the pre-anesthesia unit without receiving their adjusted scheduled dose of long-acting or intermediate-acting insulin. This is especially important in patients with type 1 diabetes who are traditionally at higher risk of diabetic ketoacidosis if insulin regimen is disrupted.

Intraoperative: Upon arrival to the pre-anesthesia, diabetes management is largely dependent on patient's type of diabetes, blood glucose upon arrival, and the type of surgery. Target blood glucose range in the perioperative period is 80–180 mg/dL [11]. Tighter perioperative glycemic control does not improve outcomes and has been associated with hypoglycemia [51].

Minor surgeries: Patient's blood glucose upon arrival to the pre-anesthesia unit can determine treatment and BG monitoring frequency as outlined in Table 6.3. Patients who have a blood glucose level >180 mg/dL and are not responding to subcutaneous insulin within an hour can be started on IV insulin infusion. On the other hand, patients with blood glucose lower than 100 mg/dL should be started on IV dextrose infusion as outlined in Table 6.8. All other patients should receive maintenance intravenous fluids that do not

Table 6.7 Preoperative diabetes management night before or morning of surgery

Diabetes medication management
• **Long-acting (glargine or detemir) insulin:** Inject 80% of scheduled dose at bedtime or in the morning before surgery depending on the patient's usual administration time
• **Intermediate-acting (NPH) insulin:** Inject ½ of usual dose
• **Rapid (aspart, lispro, glulisine) or short-acting (regular) insulin:** Omit morning dose (including inhaled insulin)
• **Premixed insulin (70/30, 75/25, 50/50):** Inject ½ of the NPH component of the usual premixed insulin and no rapid or short-acting insulin on the morning of surgery
• **Oral and non-insulin injectable diabetes medications:** Discontinue on the morning of surgery [9]
Blood glucose monitoring
• Check blood glucose at bedtime and on the morning of surgery, and every 4–6 h thereafter
• If hypoglycemic at bedtime or overnight, patient should be treated with glucose gel and not by juice

Table 6.8 Intraoperative diabetes management for nonmajor surgery

BG < 80 mg/dL ↓ Give at least100 mL D10W IV or 25–50 mL(1/2–1 amp) of D50 ↓ Check BG in 15–30 min	BG 80–100 mg/dL ↓ Begin D5W at 40 mL/h or D10 W at 20 mL/h Check BG in 1 h ↓ Check BG in 1 h	BG 101–180 mg/dL ↓ Continue to monitorBG every 2 h	BG > 180 mg/dL ↓ Give corrective rapid-acting insulin q4hrs (Table 6.3) or start Insulin infusion, check BG every hour

contain dextrose such as lactated ringers, normal saline, or 0.45% normal saline.

Major surgeries: It is recommended that IV insulin infusion be started for patients undergoing chest, abdominal cavity, vascular bypass, transplant, spinal or brain surgery, total hip or knee replacement surgeries, or a surgery anticipated to last longer than 4 h. For patients who are started on IV insulin infusion, a dextrose containing intravenous fluid is necessary. D5W at 40 mL/h or D10W at 20 mL/h should be started to provide approximately 50 g of glucose over 24 h.

Postoperative: While patient is in the post-anesthesia unit, management and frequency of BG monitoring remains similar to during surgery (Table 6.8). If patient's BG is greater than 180 mg/dL, blood glucose should be checked hourly. Corrective dose of rapid-acting insulin should be administered every 4 h. Upon arrival to the regular floor, it is recommended to start basal plus nutritional or basal plus corrective rapid-acting insulin regimen [52, 53]. If patient is not eating, nutritional insulin should be held. It may start later at reduced doses based oral nutrition intake [9, 53]. Patients who are status post cardiac surgery should continue on IV insulin infusion.

Hyperglycemia Management of the Critically Ill Inpatient

It is well established that mortality, morbidity, and length of stay increases when blood glucose levels rise above 180–200 mg/dL in critically ill patients [5, 9]. More recently it has been established that hypoglycemia in these patients is also associated with increased mortality. It is therefore important to have a form of insulin that both acts and clears rapidly in order to quickly correct and prevent hyperglycemia and hypoglycemia. When regular insulin is injected by intravenous (IV) versus subcutaneous (SC) routes, peak serum levels are reached within 2 min by IV route versus 60 min by SC route resulting in peak glucose lowering at 15 min by IV route versus 180 min by SC route. Rapid glucose lowering by IV insulin is coupled with rapid insulin clearance allows blood glucose levels to return to baseline 30 min post injection if the insulin infusion is stopped [54–56]. The slower performance of SC administered regular insulin is because regular insulin is crystalized around a zinc molecule in the shape of a hexamer. It takes time for this hexamer to dissociate into first dimers, and then monomers form which rapidly crosses the capillary membrane and binds to insulin receptors. Thus, IV insulin infusions are the standard of care in critically ill patients. Exceptions are patients who are predicted to be discharged from the ICU in less than 24 h. Those patients may start or continue SC insulin as previously discussed.

Target blood glucose range: The current blood glucose recommendations for critically ill patients by the American Diabetes Association (ADA) in conjunction with the American Association of Clinical Endocrinologists (AACE) [9] and separately by the Society of Critical Care Medicine [57] are listed in Table 6.9. In order to understand the rationale behind these recommendations we will briefly review the landmark randomized controlled trials leading to them. The Leuven trial in 2001 [58] was a single center trial that compared BG target of 80–110 mg/dL versus 180–200 mg/dL in surgical ICU. It showed 42% reduction in mortality and 34% reduction in length of stay. The Leuven group repeated their study in medical ICU but were not able to show similar reduction in mortality. In fact, there was a trend toward increase mortality that was found to be strongly associated with hypoglycemia [59]. The VISEP study [60] compared the two target BG range groups defined by the Leuven trials [58, 59], but in patients with septic shock. The study reported significant increase in adverse events (11% vs. 5%) in the 80–110 mg/dL group versus the 180–200 mg/dL group and the study was stopped early due to significantly increased rate of hypoglycemia (17% vs. 4%) in the tightly controlled group. The NICE-SUGAR study [61], a large, multi-national study, compared a target range of 81–108 mg/dL to 140–180 mg/dL in both surgical and medical ICUs. The trial showed significant increase in 90-day mortality. This increased mortality was shown to be associated with hypoglycemia, although no causal relationship was established [62]. Of note, this was the only study that had a comparison group with blood glucose levels below 180 mg/dL, which is well below the 200 mg/dL threshold that prior studies had shown to increase morbidity and mortality. It is worth noting that the safety of blood glucose levels between 110 mg/dL and 140 mg/dL is still unanswered. The ADA/AACE recommendations [9] aim to keep the lower end of their target higher enough (140 mg/dL) to preemptively prevent less experienced ICU teams from entering their patients to the danger blood glucose zone of <110 mg/dL, which was associated with higher mortality as shown in the NICE-SUGAR study [61], and an upper end of the range <180 mg/dL to avoid falling into the >200 mg/dL "danger zone." It is recommended that blood glucose should be kept in the lower end of this range [9]. However, certain hospitals with lower hypoglycemia rates have chosen tighter target ranges, such as 120–160 mg/dL, presuming the unexamined 110-140 mg/dL to be safe, and trying to keep their upper target range away from 200 mg/dL.

For patients who are status post cardiac surgery, the Society of Critical Care Medicine recommends a target range of 100–150 mg/dL [57] (see Table 6.9). However, tight control (100–140 mg/dL) on IV insulin infusion in

Table 6.9 Glucose targets in critically ill patients with and without diabetes

Established diabetes	No diabetes • Status post cardiac surgery or • Status post ischemic cardiac or neurological event
140–180 mg/dL	100–150 mg/dL

post cardiac surgery patients has shown to lower adverse outcomes for patients without diabetes. Patients with diabetes have no increased complications in the 140–180 mg/dL target group when compared to the 100–140 mg/dL target group [63, 64]. Other patients without diabetes who may benefit from tighter glycemic control are those who are admitted for an acute ischemic cardiac [65] or neurological event, provided these targets can be achieved without significant hypoglycemia [57].

Effective insulin infusion protocols must use dynamic as opposed to static algorithms that use the last blood glucose, the rate of change in blood glucose, as well as the current insulin infusion rate when recommending the new insulin infusion rate [11]. This will help prevent hyperglycemia if rate of correction is too slow, and prevent hypoglycemia if the rate of correction is too fast. Many different paper-based and computer-based dynamic algorithms are available, and no single protocol or algorithm has been established as the most effective for achieving and maintaining glucose targets or achieving lowest hypoglycemia rates [66, 67]. It is important that the hospital's chosen protocol is validated, and has demonstrated safety and efficacy [67]. Key elements of an intravenous insulin infusion protocol are listed in Box 6.3 [66–68]. In general, a potential hypoglycemic or hyperglycemic scenario should be anticipated and proactively addressed with clear guidelines in the protocol. For example, in the event of abrupt TPN/PPN, steroid, or vasopressor discontinuation, the infusion rate should be reduced by 50%, with resumption of blood glucose checks once every hour until blood glucoses are stable. It needs to be noted that patients with diabetic ketoacidosis and hyperglycemic hyperosmolar syndrome will need modified insulin infusion protocols that prevents rapid correction of hyperglycemia.

Box 6.3: Key Elements of an Intravenous Insulin Infusion Protocol

1. Clear instructions on the criteria for initiation of IV insulin infusion
2. Clearly stated target blood glucose
3. Clear instructions on how to calculate initial IV insulin infusion rate
4. Instructions on frequency of blood glucose monitoring
5. Clear instructions on management of hypoglycemia
6. Guidance for handling situations where TPN, PPN, steroids, or vasopressors are added or removed
7. Guidance for transitioning from IV insulin to SC insulin
8. Instructions on how to change insulin infusion rate

Transitioning Off Insulin Drip

Once critically ill patients become clinically stable and ready for transfer out of the ICU, and are tolerating at least 50% of their diet, or are on a stable regimen of TPN or PPN, they are ready to come off the insulin infusion. Not all patients who were on an insulin infusion in the critical care unit will need to transition to SC insulin. Patients who need to be transitioned are those with type 1 diabetes, type 2 diabetes, or those without diabetes requiring more than 1–2 units/h of insulin [69]. The Joslin Diabetes Center guidelines for transitioning patient from intravenous (IV) to subcutaneous (SC) insulin are listed in Box 6.4 [69, 70].

Box 6.4: Guidance for Transitioning from IV to SC Insulin

1. Determine the average hourly rate of insulin over the past 8 h
2. Multiply this number by 24 to determine total IV insulin requirements in past 24 h (TDD-IV)
3. Use 60–80% [71, 72] of the total TDD-IV to derive your TDD of SC insulin (TDD-SC)
4. If the patient was NPO, the TDD-SC number is equivalent to the patient's basal insulin
5. If the patient was eating over the past 24 h, then ½ of the TDD-SC is bolus and the other half basal
6. Overlap IV insulin infusion for a minimum of 4 h if subcutaneous insulin glargine is given without subcutaneous fast-acting insulin

Hypoglycemia

Early recognition and treatment of hypoglycemia, utilizing a hospital-wide nurse-led protocol, significantly reduces adverse outcomes [73, 74]. Treatment depends on severity of hypoglycemic episode, and whether or not patient is conscious. The hypoglycemia management guidelines by Joslin Diabetes Center are listed in Table 6.10 [75]. Recurrence of hypoglycemia is common. In one study, 84% of patients with severe hypoglycemia had had one prior episode of hypoglycemia [11]. Failing to adjust insulin regimen after a hypoglycemic event is common [11] and is a strong predictor of recurrence of hypoglycemia and declining renal function [9]. Therefore, it is important for treating provider to review patient's insulin regimen and adjust basal or corrective bedtime insulin doses in the event of fasting hypoglycemia, or bolus and/or corrective insulin doses in the event of postprandial hypoglycemia [5]. The Joslin Diabetes Center guidelines on insulin adjustments for hypoglycemia are detailed in Box 6.5 [75]. In about 20% of cases, rebound hyperglycemia is experi-

enced after a hypoglycemic event. Close communication between physicians and nursing staff prior to making any changes to patient's insulin regimen is quite important. Over-correction with carbohydrates is frequently the main cause of rebound hyperglycemia. For example, giving no more than 20 g of carbohydrate for correction of blood glucose between 50 and 70 mg/dL and calculating D50 dose based on blood glucose reading at the time of hypoglycemic episode instead of injecting full ampule are good practice. Examples and serving sizes of simple carbohydrates used to treat hypoglycemia are listed in Box 6.5. It needs to be noted that patients with gastroparesis should receive treatment with glucose gel due to their delayed gastrointestinal absorption. Blood glucose should be checked 15 min later, and if blood glucose remains <70 mg/dL, another 15 g of simple carbohydrates should be given. As discussed in the previous section, for critically ill patients the consensus bar for hypoglycemia is considered 100 mg/dL.

Box 6.5: Examples of 15 g of Carbohydrate

- 4 glucose tablets
- 1 tube glucose gel
- 4 oz (1/2 cup of juice or regular soda)
- 4 teaspoons of sugar

Summary

During hospital admission, proactive glycemic control for critically ill and non-critically ill patients with diabetes is important to prevent hospital complications and mortality whether patients are managed in surgical or medical units. Hyperglycemia needs to be avoided with institution of long-acting basal plus nutritional and corrective rapid-acting bolus insulin, and not only by corrective regular insulin doses before sliding scale. Timely detection of hypoglycemia and nurse-led management protocols have become a standard of care. Timely changes in treatment are greatly facilitated by glucose meters that are wirelessly connect to the hospitals electronic health record system, as well as by using computerized physician insulin order entry systems. This combination allows physicians to rapidly access patient's blood glucose readings from anywhere in the hospital and immediately intervene. Good communication between hospital team and availability of certified diabetes educator are shown to improve diabetes control during hospital admission and ensure patient safety after discharge. As tight glycemic control may be associated with increased hypoglycemia risk, further studies are still needed to determine ideal blood glucose targets for both critically ill and non-critically ill patients. With increasing attention to medication errors and iatrogenic complications in the hospital setting, safely achieving euglycemia will be of paramount importance.

Table 6.10 Hypoglycemia management (non-critically ill patients)

Treatment		
Conscious on oral feeding	BG: 50–69 mg/dL	15–20 g of simple carbs
	BG <50 mg/dL	20–30 g of simple carbs
Conscious but NPO	On IV insulin	• Stop insulin infusion • Inject bolus dose D50W IV. Dose in mL = (100-BG) × 0.4 • Start D10W IV at 25 cc/h. • Once BG is back to >100 mg/dL, stop D10W and resume insulin infusion at 50% of the previous rate
	On SC insulin	• Inject bolus dose D50W Dose in mL = (100-BG) × 0.4 • Start D10W IV at 25 cc/h • Once BG is back to >100 mg/dL, stop D10W and resume insulin regimen after appropriate adjustments are made
Unconscious	No IV access	• Give 1 mg glucagon IM or 0.5 mg for patients <50 kg body weight • Once IV access is established, proceed with steps outlined for conscious patient
Insulin adjustment		
Fasting hypoglycemia	• Reduce long-acting basal insulin by 20% if BG is 50–70 mg/dL • Reduce long-acting basal insulin by 30% if BG is <50 mg/dL • If patient received corrective insulin prior to the event, consider increasing sensitivity factor (SF) of corrective insulin	
Postprandial hypoglycemia	• Reduce bolus (nutritional) insulin by 20–50% for the duration that patient's oral food intake is below baseline • If patient had received corrective insulin prior to the event, consider increasing sensitivity factor (SF) of corrective insulin	

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Part II

Pathophysiology

Physiology and Pathophysiology of Wound Healing in Diabetes

7

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Abstract

Wound healing is a dynamic process comprising of overlapping phases of hemostasis, inflammation, proliferation, and remodeling that involve multiple cell types. This highly organized and coordinated series of processes result in the restoration of tissue integrity. Deregulation in any of these processes leads to a delayed or nonhealing phenotype as seen in diabetic foot ulcers (DFUs). The functions and cell-to-cell communication between different cell types contributing to wound healing (keratinocytes, fibroblasts, endothelial cells, neutrophils, and macrophages) and their deregulation in chronic nonhealing ulcers are discussed in detail. The balance of signaling factors, including growth factors and gene expression regulators such as microRNA, and their spatiotemporal control is indispensable for successful wound healing, while their dysregulation contributes to pathophysiology of DFUs. Additional factors that contribute to the delayed healing seen in diabetes include macro- and microvascular, neuropathic, immune functions, and microbiome abnormalities. Novel therapeutic approaches including cell therapy, stem cells, and micrografting that provide perspective on how to efficiently treat patients with DFUs are also discussed.

Abbreviations

AGE	Advanced glycation end product
ASC	Adipose-derived stem cell
BM-MNC	Bone marrow-derived mononuclear cell
BM-MSC	Bone marrow-derived mesenchymal stem cell
CEA	Cultured epithelial autograft
DFU	Diabetic foot ulcer
EC	Endothelial cell
ECM	Extracellular matrix
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGR3	Early growth response factor 3
En1	Engrailed-1
EPC	Endothelial progenitor cell
FGF	Fibroblast growth factor
GM-CSF	Granulocyte macrophage colony stimulating factor
HB-EGF	Heparin-binding EGF
HBO	Hyperbaric oxygen
IGF-1	Insulin-like growth factor 1
IKBKB	Inhibitor of nuclear factor kappa-B kinase subunit beta
IL-1	Interleukin-1
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
iPSC	Induced pluripotent stem cell
KGF	Keratinocyte growth factor
LepR	Leptin receptor
MAPC	Multipotent adult progenitor cell
miR	Micro-RNA
mKitL	Membrane-bound Kit ligand
MMP	Matrix metalloproteinase
MSC	Mesenchymal stem cell
NET	Neutrophil extracellular trap
NLRP	Nod-like receptor protein
NO	Nitric oxide
Nrf2	Nuclear factor like 2
PDGF	Platelet-derived growth factor

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PPARγ	Peroxisome proliferator-activated receptor γ
rhEGF	Recombinant human epidermal growth factor
rhPDGF	Recombinant human platelet-derived growth factor
rhVEGF	Recombinant human vascular endothelial growth factor
sKitL	Soluble Kit ligand
TGF-ß1	Transforming growth factor-beta 1
TGF-α	Transforming growth factor alpha
TIMP	Tissue inhibitor of metalloproteinase
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor
WEE1	Wee1-like protein kinase

Physiology of Wound Healing

Wound healing is an evolutionarily conserved process that aims to restore the damaged epithelial barrier between the body and the outside world. This complex process involves many cellular responses including inflammation, proliferation, migration, angiogenesis, and tissue remodeling. Immediately after the injury, blood components are released into the wound site, activating the clotting cascade. The resulting clot induces hemostasis, releases chemotactic cytokines, and provides a matrix for the influx of inflammatory cells. Inflammation is characterized by leukocyte migration and arrival to the site of injury. Neutrophils arrive first to remove contaminating bacteria and release pro-inflammatory cytokines [1]. They are followed by monocytes, which differentiate into macrophages at the site of tissue injury. Macrophages play an important role in augmenting the inflammatory response and removing nonviable tissue. At the same time, many different cell types respond to initial inflammatory signals and migrate to the wound site, including keratinocytes, endothelial cells, and both circulating and local progenitor cells. Once they arrive and proliferate, the processes of reepithelialization, neovascularization, and granulation tissue formation commence. Granulation tissue formation begins during the inflammatory phase, forming a "beefy red" and highly vascular region of the healing tissue, predominantly relying on neovascularization [1, 2]. As the wound closes, the immature fibrin matrix and granulation tissue are replaced by collagen and scar.

Wound healing as a process does not end at wound closure, although this is the visible sign of complete healing. After closure, the remodeling phase begins, which is characterized by continuing collagen deposition and cross-linking. During remodeling, balance is established between collagen synthesis and degradation, which gives the scar its tensile strength [1, 3]. Wound healing in adults results in scar formation, fibrosis, and contracture. However, fetal skin, up to midway through the third trimester, heals without scar formation, using a unique regenerative pathway [4].

Cellular responses to injury involve direct cell–cell and cell–matrix interactions, as well as indirect cross talk between different cell populations via soluble mediators. Thus, wound healing is orchestrated through the integration of multiple signals (growth factors, cytokines, and chemokines) released by participating cells including keratinocytes, fibroblasts, endothelial cells, neutrophils, macrophages, and platelets. The appropriate balance of these signaling factors as well as their spatiotemporal control is essential for successful wound healing [5–9]. The functions of various contributing cells: keratinocytes, fibroblasts, endothelial cells, neutrophils, and macrophages are discussed in more detail below.

Cellular Components of Wound Healing

Keratinocytes

Keratinocytes play several critical roles in the wound healing process and are among the most important cells that respond to injury and accelerate healing. Under normal conditions, their main role is to form the barrier of the skin. Once the skin is wounded, keratinocytes play many important roles, including the release of cytokines and growth factors, which recruit other cell types, stimulate matrix formation, and promote angiogenesis [10, 11]. Simultaneously, keratinocytes also migrate and proliferate within the wound bed to accelerate closure and restore the skin barrier [9].

In healthy skin, keratinocytes proliferate in the basal cell layer and differentiate in the suprabasal layers. Basal keratinocytes are mitotically active and help form the basement membrane by promoting cross talk with dermal fibroblasts, melanocytes, and Langerhans cells. Once keratinocytes migrate above the basal cell layer, they change phenotypically and begin to differentiate. During this process, keratinocytes stop dividing, change their keratin production from K5/K14 to K1/K10, and begin producing a number of other insoluble proteins [12]. Terminal differentiation results in loss of nuclei and protein cross-linking, giving rise to a cornified layer that forms the epidermal barrier [9, 13, 14]. The perpetual process of keratinocyte differentiation and upward migration maintains a strong barrier to the outside world.

Because keratinocytes are responsible for barrier maintenance, they are equipped for rapid response to injury. When the epidermal barrier is disrupted, keratinocytes release prestored interleukin-1 (IL-1), which is the first signal that alerts nearby cells to barrier damage [10, 15]. In addition to the common initiator, IL-1, certain cytokines and growth factors such as tumor necrosis factor alpha (TNF-α) and epidermal growth factor (EGF) are released by keratinocytes that

together with IL-1 act in both an auto- and paracrine manner [9, 16–19]. This process, termed the "keratinocyte activation cycle," is characterized by changes in cellular behavior (migration, proliferation), induced secretion of multitude of other growth factors and cytokines, and expression of K6, K16, and K17 keratin proteins, which are often considered as the first markers of epidermal healing [20, 21].

To close a breach in the epidermal barrier, keratinocytes at the wound edge first loosen their adhesion to each other and the basal lamina. Additionally, keratinocytes display remarkable flexibility, which allows migration over the extracellular matrix (ECM) deposited by activated dermal fibroblasts. This process is facilitated by rearrangement of integrin receptors and reassembly of the associated actin cytoskeleton and keratin filament network [14]. Growth factors and cytokines such as EGF, keratinocyte growth factor (KGF), transforming growth factor alpha (TGF-α), fibroblast growth factor (FGF), interleukin-1 (IL-1), and interleukin-6 (IL-6) have been shown to be crucial regulators of keratinocyte proliferation, migration, and reepithelialization as well as communication with other cell types [7, 10, 15].

First, the migrating epithelial tongue advances to cover the wound with a thin layer. Then, keratinocytes proliferate to ensure an adequate supply of cells to encase the wound. Once the wound is healed, defined as being fully epithelialized with no drainage and covered by a keratinocyte monolayer, the proliferation signals cease and the stratification process begins again. Thus, keratinocytes become "deactivated" and revert to their previous normal differentiation pattern.

Fibroblasts

Complex interactions and cross talk between fibroblasts, keratinocytes, and other cell types participating in wound healing is crucial for successful wound closure. Under normal conditions, fibroblasts synthesize collagen and ECM, maintaining the structural integrity of the skin. Fibroblasts play a vital role in wound healing as they migrate, proliferate, and supply ECM for tissue repair. Another of the many important roles of fibroblasts is to provide contractile properties to the wound as myofibroblasts. Much like keratinocytes, fibroblasts' various roles are tightly regulated by cytokine and growth-factor signaling during the process of wound healing.

Recently, it has been shown that there are multiple lineages of fibroblasts with varying functions based on their site of origin and embryonic expression of certain genes [22]. A single lineage of fibroblasts that express the gene Engrailed-1 (En1) has been shown to cause the majority of fibrosis and scar formation during cutaneous wound healing [22]. In mice, these fibroblasts originate in the papillary dermis and migrate throughout to the lower reticular dermis in response to wounding [22]. Therefore, because the definition of fibroblasts is quite broad, it appears to include at least a few morphologically and functionally distinct phenotypes with likely more that will be characterized in the future.

Dermal fibroblasts at the site of injury begin to proliferate as an early response to wounding. A few days after wounding, fibroblasts migrate into the provisional matrix of the wound clot to lay down their own collagen-rich matrix [23–25]. This ECM acts as a "scaffold" during tissue repair, providing structural support and attachment sites for cell surface receptors while simultaneously acting as a regulated "reservoir" for signaling molecules that modulate diverse processes such as angiogenesis, cell proliferation, cell migration, and inflammation [26, 27]. In order to migrate into the clot, dermal fibroblasts must downregulate their collagen receptors and upregulate integrins that bind ECM proteins such as fibrin, fibronectin, and vitronectin [28, 29]. During migration, fibroblasts sense and respond to signals coming from both their local matrix environment and from the surrounding growth factor milieu.

About 1 week after wounding, the wound clot is fully invaded by activated fibroblasts. Transforming growth factor-beta 1 (TGF-ß1) is a potent pro-fibrotic signaling molecule that with other growth factors stimulates fibroblasts to synthesize and remodel the new collagen-rich matrix [24, 25, 28]. Simultaneously, a proportion of the wound fibroblasts transform into myofibroblasts, which express α-smooth muscle actin and resemble smooth muscle cells in their capacity for generating strong contractile forces [30, 31].

Conversion from fibroblasts to myofibroblasts is mediated not only by growth factors, especially TGF-ß1 [30, 32, 33], but also by mechanical tension [34–37]. Myofibroblasts align parallel to mechanical tension building up in the granulation tissue, and their appearance in the wound coincides with a strong induction of contractile properties. Collagen-gel models have been useful for the study of various tensile forces acting on and exerted by wound fibroblasts before, during, and after their contraction. Several growth factors present in the wound site and ECM reservoir are stimulators of fibroblast-driven gel contraction, which can presumably induce granulation tissue contraction in vivo [38, 39]. Platelet-derived growth factor (PDGF)-AA and -BB isoforms and TGF-ß1 lead to efficient collagen–gel contraction and can be considered stimulatory [38–41], while IL-1α and IL-1ß were shown to be inhibitory, which is due to increased matrix metalloproteinase activity [42, 43].

Contraction stop signals have been studied in a similar manner by releasing mechanically stressed anchored gels from their substrate attachments, which simulates the loss of resistance after a wound has closed. Within minutes of release from resisting forces, PDGF and EGF receptors on the cell surface become deactivated [44], and the relaxed

cells return to a quiescent state similar to that existing before the injury. Fibroblasts present in the granulation tissue undergo apoptosis, triggered by TGF-ß1 and FGF at the injury site, after wound contraction has ceased [45, 46]. Together, these mechanisms promote a return to the normal physiologic state and location of fibroblasts after the process of healing has completed.

Given the importance of fibroblasts and keratinocytes in proper wound healing, human skin substitutes have been developed as a wound treatment modality. Several cell therapies are approved for use in diabetic foot ulcers (DFUs), and two in particular utilize fibroblasts. Graftskin (Apligraf) is a human skin equivalent composed of a dermal layer containing human fibroblasts and connective tissue and an epidermal layer consisting of keratinocytes [47]. Similarly, Dermagraft is a human dermal substitute consisting of cryopreserved human fibroblasts, ECM, and a bioabsorbable scaffold [48]. Thus, fibroblasts and keratinocytes are vitally important for maintenance of the epidermal barrier as well as the process of wound healing after injury while also showing great promise in their practical translation to the bedside. Please see treatments section for additional information.

Endothelial Cells (ECs)

Local ECs are additional responders to the wound healing signals released by keratinocytes and fibroblasts. Normally, ECs are located in the wall of the vascular lumen forming the tubular structure of blood vessels and the barrier between blood and extravascular tissue. They express integrins and other cell adhesion molecules to allow selective permeability between these two compartments and are highly upregulated during angiogenesis [49, 50]. The process of angiogenesis is facilitated by growth factors, cytokines, cell–cell and cell–matrix interactions, and even exosomes from certain stem cells that activate these ECs [51]. These activated ECs along with platelets, macrophages, and fibroblasts release pro-angiogenic cytokines that lead to the invasion and migration of ECs into the ECM, EC proliferation, and new immature vascular formation [52, 53]. Before angiogenesis can begin, ECs must detach from neighboring ECs primarily by digesting the basement membrane and components of the ECM [53, 54]. This is achieved by proteolytic enzymes, including serine proteases, urokinase plasminogen activator, and matrix metalloproteinases (MMPs) that are released by activated ECs [55]. The addition of an MMP synthetic inhibitor to EC cultures significantly decreases angiogenic activity [55] highlighting the importance of this group of enzymes to EC function. Once they are liberated, ECs migrate to the site of new vessel formation via vascular endothelial growth factor (VEGF)-stimulated chemotaxis where they will proliferate [53, 55, 56]. Additionally, specific adhesion molecules, especially integrins, mediate endothelial cell–matrix interactions to ensure migration to the site of new vessel formation [53, 56].

Neutrophils

Inflammation is a key process in normal wound healing and is tightly regulated both temporally and spatially by multiple cell types. Immediately following injury, activation of the clotting cascade ensues causing platelet aggregation and leading to the formation of a fibrin clot which initiates hemostasis. These processes are important to stop blood and fluid loss as well as to provide a provisional matrix that facilitates the infiltration and recruitment of inflammatory cells and other cells to the injury site [1, 57]. Platelets degranulate and release a variety of growth factors and cytokines that act as chemoattractants for cells such as neutrophils, macrophages, endothelial cells, fibroblasts, and keratinocytes resulting in the initiation of the inflammatory phase [1, 58]. The first inflammatory cells to migrate to the wound site are the neutrophils which are the predominant cell type during the first 2 days. Their role is to remove dead cells and infectious microorganisms by phagocytosis and generation of reactive oxygen species or by releasing neutrophil extracellular traps (NETs) [59–61]. Neutrophil activity has to be rapid to minimize host tissue damage and excessive inflammation and the cells undergo apoptosis or die by NETosis once infection is under control [62]. After a few days, neutrophil infiltration ceases and expended neutrophils are phagocytosed by macrophages.

Macrophages

Macrophages appear at the wound site within 2 days after injury and play an important role in clearing matrix, cell debris, and microorganisms. Both inflammatory monocytes, recruited from the bone marrow that later become macrophages, and resident macrophages are recruited to the wound site [63, 64]. These macrophage populations change their expression profiles according to cytokine and growth factor stimuli [65, 66]. During normal wound healing, macrophages transition from a pro-inflammatory or "M1" phenotype to a wound healing-associated or "M2" phenotype [67, 68]. M1 macrophages dominate earlier during injury and express pro-inflammatory mediators and cytokines including TNF-α, IL-1, IL-6, IL-12, and inducible nitric oxide synthase (iNOS) whereas M2 macrophages dominate later during injury and express anti-inflammatory genes as well as promote ECM synthesis and cell proliferation [68–70]. Macrophages also release a battery of growth factors, chemokines, and MMPs

[64], which all aid in driving cell proliferation and ECM synthesis.

Inflammatory cells also exert their influence on the surrounding tissue by generating nitric oxide (NO) and large amounts of ROS [71]. NO and ROS are known to drive certain aspects of repair [72, 73] but, at the same time, affected wound cells must protect themselves by detoxifying programs [71, 74]. NO is a very transitory molecule, whose levels together with inducible NO synthase (iNOS) activity shows a distinct time course during normal healing [75, 76]. Although the issue of whether inflammatory cells are an essential requirement for repair remains controversial, it is clear that these cell populations exert a profound influence on all other cells within the wound and in the surrounding tissue. One of the important roles of inflammatory cytokines is to regulate angiogenesis, which they accomplish in concert with signals from other wound cells and from serum (see Section on angiogenesis). However, nonhealing wounds fail to progress through the normal phases of wound repair, but instead remain in a chronic inflammatory state. Imbalances in wound proteases and their inhibitors in chronic wounds, because of sustained production of inflammatory mediators and influx of inflammatory cells, prevent matrix synthesis and remodeling, essential for progression to a healed wound [77–82].

The inflammatory phase of wound healing has been studied in detail, but most of the research efforts were focused on onset of inflammation and little is known about inflammation resolution. Better understanding of how inflammation resolves will provide a basis for novel treatment modalities favoring the closure of chronic wounds.

Pathophysiology of Wound Healing in Diabetes Mellitus

Over 415 million people worldwide have diabetes with an estimated 29.1 million affected people in the United States alone. These numbers incur annual costs of more than $245 billion rendering it a major public health and socioeconomic concern [83]. The prevalence of diabetes is on an upward trend, possibly affecting one-third of the US adult population by 2030 [84]. Diabetes has many devastating complications, one of which is DFUs, which occurs in 15% of diabetic patients often leading to lower-limb amputations [1]. Following amputation, DFU patients have a 5-year mortality rate of nearly 50% [85]. DFUs often lead to lengthier hospitalization with associated high treatment costs, pain, reduced quality of life [86, 87] and are a significant cause of morbidity and mortality [88].

Wound healing is a dynamic process comprising of overlapping phases of hemostasis, inflammation, proliferation, and remodeling that involve multiple cell types. This highly organized and coordinated series of processes results in the restoration of tissue integrity and functions. Deregulation in any of these processes leads to a delayed or nonhealing phenotype as seen in DFUs [1, 85]. Factors that contribute to the delayed wound healing seen in diabetes are multifactorial and include macro- and microvascular, neuropathic, immune functions, and biochemical abnormalities. Indeed a recent study provided evidence that additional diabetic-associated problems may play an important role in the development of DFUs since only subtle changes were found between non-ulcerated non-neuropathic diabetic foot skin and healthy nondiabetic foot skin using comparative genomic analyses as well as detailed histolomorphological evaluations [89]. Poor glucose control and hyperglycemia are additional factors that may contribute to the metabolic pathophysiology of diabetes-related complications [90, 91]. DFU development involves both extrinsic and intrinsic factors. Extrinsic factors include callous formation, excessive pressure, repeated trauma, and wound infection [92], while intrinsic factors that contribute to an impairment in diabetic wound healing include prolonged inflammation, persistent infection, imbalanced proteolytic activity, improper formation and remodeling of the ECM, reduced growth factors, poor angiogenesis and various cell type and stem cell dysfunction, cellular senescence and reduced reepithelialization [1, 93–96]. Prolonged hypoxia [97] and reduced levels of neuropeptides [98] have all been shown to contribute to the impaired wound healing in DFUs.

Keratinocytes' and Fibroblasts' Role in Impaired Wound Healing

Upon cutaneous injury, epidermal keratinocytes become activated. However, this activation is not properly executed in chronic wounds and migration and proliferation of these cells are affected as a result. The EGF family, particularly family members involved in wound healing such as EGF, heparin-binding EGF (HB-EGF), and TGF-α, can bind and activate the EGF receptor (EGFR), thus leading to stimulation of keratinocyte migration and proliferation [15]. Lee et al. (2005) showed that EGF-mediated migration is blocked by glucocorticoids and this occurs through repressing K6/K16 transcription [99]. In the nonhealing edge of chronic wounds, EGF receptor (EGFR) is expressed in the cytoplasm of keratinocytes instead of the membrane as with normal epidermis [100] suggesting that these cells are unable to respond to EGF ligands. This may be a likely reason why topical application of EGF, in an attempt to heal human chronic wounds, is met with limited success [101]. Nonhealing ulcer keratinocytes are hyperproliferative in both basal and suprabasal layers of the epidermis giving rise to parakeratosis and hyperkeratosis, indicating impaired differentiation (Fig. 7.1) [102, 103]. The differentiation markers K1/K10, filaggrin,

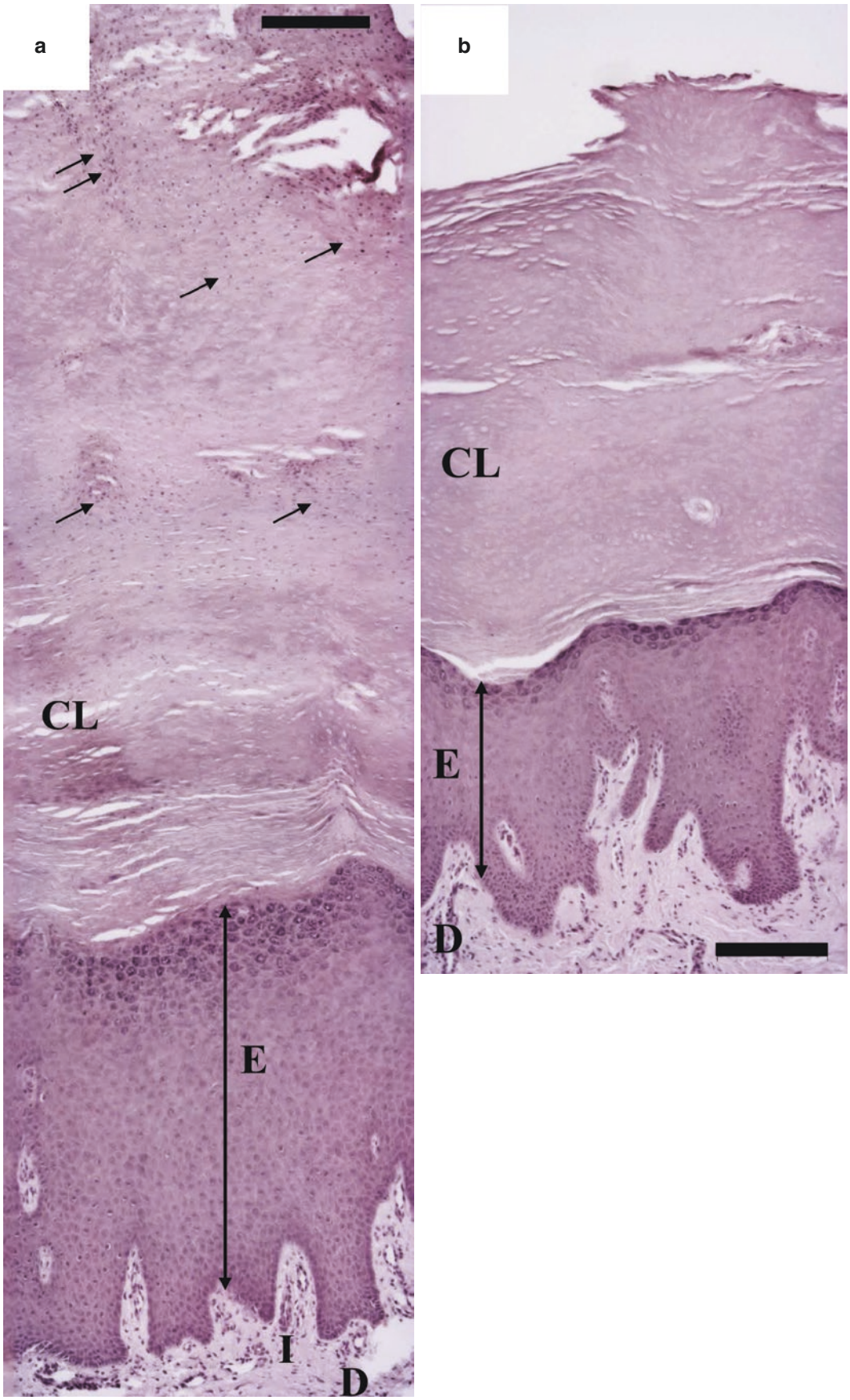

Fig. 7.1 Histology of DFU (**a**) compared to adjacent non-ulcerated diabetic foot skin (**b**). DFUs display a hyperproliferative nonmigratory epidermis with parakeratosis. Significantly thicker cornified layer (hyperkeratosis) and the presence nuclei in the cornified layer (parakeratosis) are characteristic for DFUs (**a**), and not present in adjacent non-ulcerated diabetic skin (**b**). *E* epidermis, *D* dermis, *CL* cornified layer, *I* increased cellular inflammatory infiltrate; arrows indicate nuclei in the cornified layer. Scale bar = 200 μm

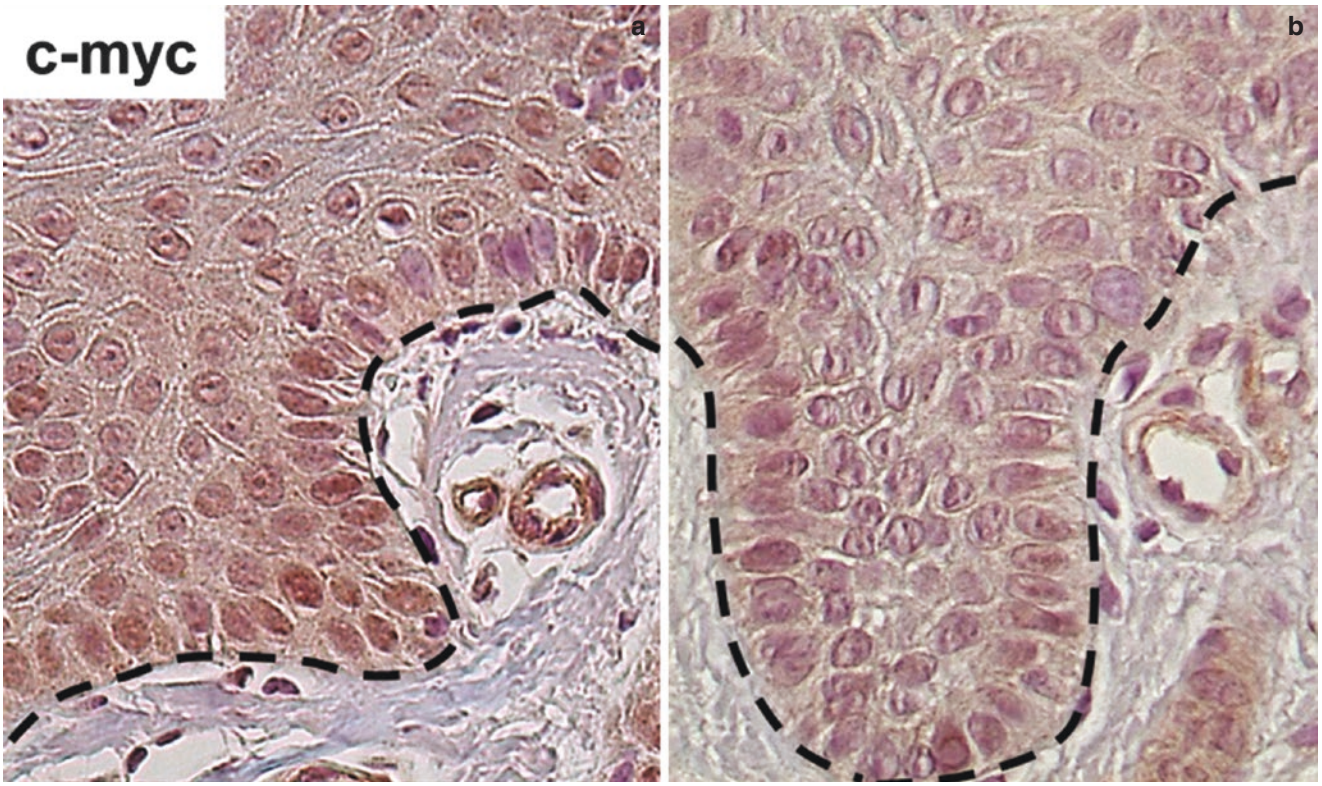

Fig. 7.2 Wound edge of the chronic DFU shows overexpression of c-myc. Immunohistochemistry with c-myc-specific antibody of nonhealing (**a**) and healing DFU (**b**). Nuclear presence and overexpression of c-myc (brown) contributes to hyperproliferative phenotype in the nonhealing DFU. Healing DFU (**b**) has less c-myc positive nuclei

and a subgroup of small proline-rich proteins were found to be suppressed while the late differentiation markers transglutaminase 1 and involucrin were induced in nonhealing venous ulcers [103]. In a prospective clinical study, it was shown that the nonmigratory and hyperproliferative phenotype of keratinocytes seen at the nonhealing wound edge of chronic ulcers was attributed to nuclear presence and overexpression of c-myc and β-catenin (Fig. 7.2) contributing to epidermal stem cell depletion [93, 104]. In addition, stabilization of nuclear β-catenin inhibited wound healing and keratinocyte migration by blocking EGF response, inducing c-myc, and repressing K6/K16 in vitro [104]. Moreover, the epidermis of chronic nonhealing wounds has been shown to exhibit decreased expression of the precursor of the alpha3 chain of laminin [105], thus affecting cell migration.

Dermal fibroblasts from diabetic skin closely resemble those in healthy foot skin, suggesting that diabetes itself does not impact function of fibroblasts prior to wounding [89]. Namely, study of the mRNA and micro-RNA (miRNA) expression profiles of diabetic and healthy, nondiabetic foot skin fibroblasts has revealed no significant differences in gene expression levels [89].

However, fibroblasts from diabetic foot ulcers exhibit major changes including altered morphology, ECM deposition, increased apoptosis, diminished response to growth factors, reduced proliferation, and reduced migration [31, 96, 106–108]. Patient-derived fibroblasts from DFUs seeded into three-dimensional models led to decreased stimulation of angiogenesis, impaired ECM synthesis, and recapitulated the nonmigratory and hyperproliferative epidermal phenotype in organotypic cultures in the presence of keratinocytes [107]. In a study that used genomic approaches to analyze the pathophysiology of DFU fibroblasts, miR-21-5p, miR-34a-5p, and miR-145-5p were found to be induced in DFU-derived fibroblasts contributing to inhibition of cell migration and proliferation, as well as induced differentiation and cell senescence [96].

Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases

An imbalance between ECM protein synthesis and remodeling by MMPs and the tissue inhibitors of metalloproteinases (TIMPs) is seen in DFUs. Increased MMP production causes ECM degradation [109]. MMPs 1, 2, 8, 9, 14, and 26 are highly expressed in DFUs [110–113] with concomitant reduction in the expression of their inhibitors [77]. High MMP9 and a high MMP9/TIMP1 ratio has been shown to be a predictor of poor wound healing [112], whereas a higher MMP1/TIMP1 ratio is correlated with healing [114] (Fig. 7.3). Lobmann and colleagues (2002) showed an

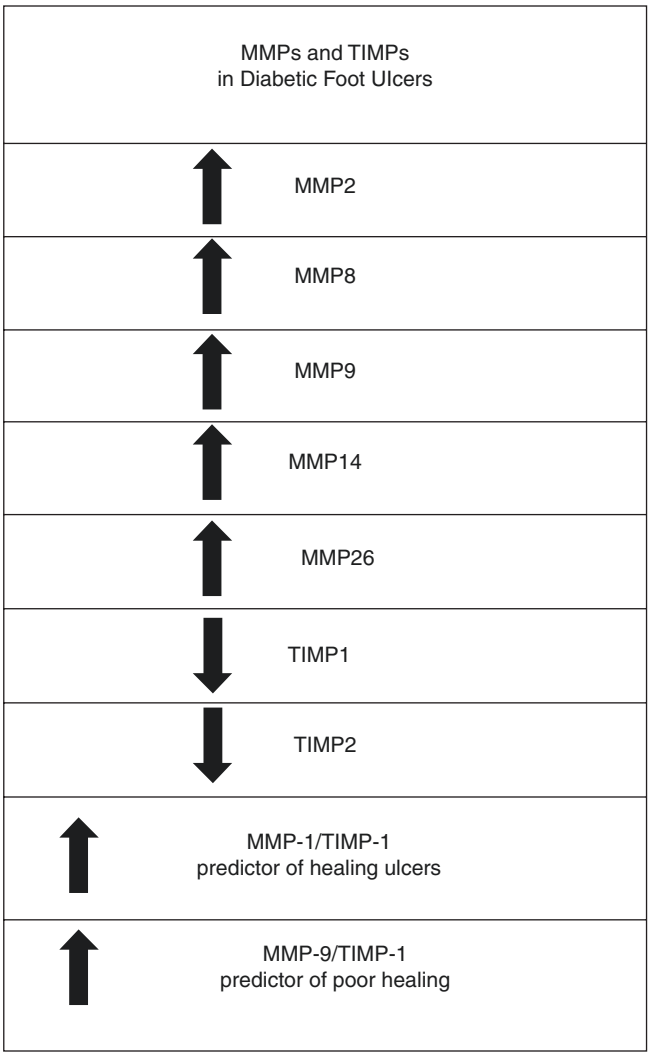

Fig. 7.3 Deregulation of MMPs and TIMPs in DFUs. Schematic overview of upregulated (arrows up) and downregulated (arrows down) MMPs, TIMPs and their ratios in DFUs.

increase in MMPs with reduced concentrations of TIMP-2 in patients with DFUs, compared to traumatic wounds of non-diabetic patients, suggesting that the increased proteolytic environment reduces ECM formation and contributes to the failure of diabetic wounds to heal [115]. The rise in MMP activity not only causes matrix degradation, which delays cell migration and inhibits collagen deposition, but also breaks down growth factors and their target cell receptors [15, 115].

Other common causative factors for chronic wounds include deregulation of certain cytokines, growth factors and their receptors and corresponding signaling molecules. Examples of these include TGF-β, FGF, insulin-like growth factor 1 (IGF-1), interleukins, VEGF, TNF-α, PDGF, EGF, EGFR, granulocyte-macrophage colony stimulating factor (GM-CSF), and receptors such as TGF-β receptors, EGFR, and bone morphogenetic protein receptor [10, 15, 93, 116].

The Role of MicroRNAs in Impaired Wound Healing

Another class of regulatory molecules, microRNAs (miRNAs), also play important role in acute and chronic wound healing. MiRNAs are small, noncoding RNAs that regulate gene expression posttranscriptionally binding to the 3' UTR of mRNAs, resulting in repression of mRNA translation or degradation [117]. As central regulators of gene expression, miRNAs have been shown to regulate various pathogenic processes of skin diseases like hypertrophic scarring and psoriasis [118, 119]. MiRNAs can also be secreted by different cells and transported via exosomes into the circulation [120, 121], where they modulate the activity of target cells. In a recent study, exosomes derived from bone marrow-derived mesenchymal stem cells contained an enrichment of distinctive miRNAs [122] with the ability to enhance proliferation and migration of both normal and diabetic wound fibroblasts [51]. Furthermore, aberrant expression of miRNAs can contribute to inhibition of healing in chronic wounds [123–125]. In nonmigratory epidermis of chronic venous ulcers, expression of miR-16, miR-20a, miR-21, miR-106a, miR-130a, and miR-203 was found to be altered [125]. Overexpression of miR-21 and miR-130a inhibited epithelialization in a human ex vivo wound model and in an in vivo rat wound model via direct targeting of Leptin Receptor (LepR) and Early Growth Response Factor 3 (EGR3) [125]. Similarly, miR-198 expression has been shown to persist in DFUs [126]. Using gene expression analysis, it was reported that induction of miR-15b-5p in DFUs deregulated DNA repair mechanisms and inflammatory response by targeting inhibitor of nuclear factor kappa-B kinase subunit beta (IKBKB) and Wee1-like protein kinase (WEE1) genes, respectively [127]. Studies on diabetic mouse wounds have also reported reduced expression of miR-146a [128] and upregulation of miR-203, miR-483-3p, and miR-210 [129, 130]. Moreover, hypoxia-induced miR-203 and miR-210 expression inhibited wound closure and keratinocyte proliferation in an ischemic murine wound model [131]. Future studies aiming to identify miRNAs deregulated in patients with chronic ulcers have a potential to identify novel diagnostic and therapeutic targets.

Angiogenesis in Diabetes

During wound healing, new capillaries form and replace damaged capillaries in a process known as neovascularization. Neovascularization is important to reestablish oxygen and nutrient supply to the wound and remove waste products [53]. Angiogenesis, one form of vascularization, is the formation of new blood vessels from preexisting ones and is usually caused by tissue injury or neoplastic transformation

[132, 133]. During the proliferative phase of wound healing, endothelial cells, stimulated by VEGF, FGF2, or low oxygen tension/hypoxia, migrate to the wound and induce angiogenesis and sprout capillaries to vascularize the tissue that is being formed [134, 135]. Angiogenesis arises through a finely balanced process involving pro-angiogenic and anti-angiogenic mediators, cells ECM, cytokines and growth factors and a shift in this balance leads to impaired angiogenesis [133, 136, 137] (Table 7.1). Vasculogenesis is the de novo formation of blood vessels from bone marrow-derived endothelial progenitor cells (EPC) [138]. These newly formed vascular structures mature into capillaries, arterioles, arteries, venules, and veins.

Impaired angiogenesis and vasculogenesis, as a result of deregulation and cleavage of growth factors, and their receptors leads to insufficient oxygenation and suboptimal delivery of nutrients to the wound contributing to poor diabetic wound healing [136, 139] (Tables 7.1 and 7.2). Dysfunctional angiogenesis is also a key player in many diabetes-related microvascular complications including diabetic nephropathy, diabetic peripheral neuropathy, and diabetic retinopathy [133, 136].

VEGF, a pro-angiogenic growth factor, is important for endothelial cell proliferation and migration, ECM degradation, vessel permeability, and vasodilation [136]. Deregulated VEGF and associated signaling pathway contributes to diabetes-related pathologies [133, 136]. In some organ systems, high levels of VEGF act as a pathologic angiogenic stimulus (i.e., ocular neovascularization), while in others reduced levels of VEGF activity lead to pathology (i.e., nephropathy, peripheral neuropathy, and wound healing) [133, 136, 140, 141].

Another contributing factor to the impaired angiogenesis seen in diabetics is the bone marrow-derived EPC (Tables 7.1 and 7.2). Dysfunctional EPC, diminished EPC numbers and defective recruitment, as well as transition of EPC phenotype to a pro-inflammatory one have all been observed in diabetic patients [142–147]. Some causes of EPC dysfunction and reduced recruitment from the bone marrow in diabetic individuals include hyperglycemia, increased oxidative stress, chronic inflammation, and NADPH oxidase activation [148–150]. In diabetic murine wounds exhibiting delays in healing, defective recruitment, survival and proliferation of EPCs were also reported [151].

Table 7.1 Overview of angiogenesis in acute and chronic wound healing

Normal angiogenesis	Angiogenesis in DFU
Pro-angiogenic cytokines (including VEGF) are released from platelets, monocytes, and fibroblasts	Fibroblasts may become senescent in chronic wounds and lose their ability to provide angiogenic functions [53, 254]
Endothelial cells (ECs) disrupt their interactions with neighboring ECs	Resident ECs on the chronic wound may lose their ability to support new vessel formation [136]
ECs digest the basement membrane and extracellular matrix (ECM) components (via matrix metalloproteinases)	Impaired balance between the accumulation of ECM components and their remodeling by MMPs [115, 255]
ECs, fibroblasts, platelets, smooth muscle cells, and monocytes release more pro-angiogenic cytokines	The chronic wound environment impairs cellular proliferation and angiogenesis [256]. Impairment of leukocyte function and proliferation occur in diabetic wounds [158]
ECs invade ECM and migrate/proliferate to form new vessels	EC adhesion, migration through the ECM, and proliferation is impaired in diabetic wounds [133, 136, 257]

Table 7.2 Overview of normal vasculogenesis process and its impairment in DFUs

Normal vasculogenesis	Vasculogenesis in DFU
Multipotent adult progenitor cells (MAPCs) differentiate into hematopoietic precursor cells or early endothelial progenitor cells (EPCs) in the bone marrow	Impaired VEGF-induced proliferation response in EPCs [257]
Increased vascular endothelial growth factor-A (VEGF-A) induces vascular endothelial growth factor receptor-1 (VEGF-R1) activation and subsequently increased matrix metalloproteinase-9 (MMP-9) secretion	Hyperglycemia-mediated inhibition of VEGF [149, 257]
Increased MMP-9 mediates the conversion of membrane-bound Kit ligand (mKitL) to soluble Kit ligand (sKitL), which mobilizes EPCs from the bone marrow to circulation	Decreased number and function of circulating EPCs impairs healing [149, 150] Decreased EPCs in the bloodstream is correlated with nonhealing DFUs and can be used to predict healing potential [212]
Early EPCs in the circulation further differentiate to late EPCs and gain specific endothelial cell (EC) surface markers	Diminished blood supply to peripheral wound [149]
Late EPCs arrive to the site of new vessel formation and further differentiate into mature ECs or act as a source of pro-angiogenic cytokines	EPCs demonstrate abnormal mobilization and homing mechanisms in diabetics [149–151]

A better understanding of the mechanisms that cause dysfunctional angiogenesis is of vital importance particularly in light of the upward trend in diabetes mellitus microvascular complications. Further understanding of the role of angiogenesis in these pathologies is necessary to pave the way forward for the development of novel therapies.

Inflammation and Infection in Diabetic Wound Healing

A persistent inflammatory state contributes to the poor wound healing phenotype seen in chronic wounds and may be caused by multiple factors. Uncontrolled inflammation is known to induce MMP expression, causes tissue damage, decreases collagen synthesis, and inhibits epithelialization [92, 109, 152]. Elevated levels of advanced glycation end products (AGEs) in serum of diabetic individuals result in a subclinical chronic inflammatory state and affects synthesis of collagen [92, 152]. Hyperglycemia has been shown to elevate oxidative and inflammatory stress via ROS and tumor necrosis factor alpha (TNF-α), sustaining inflammation [153, 154]. Neuropathy and uncontrolled diabetes can affect infiltrating cell numbers in the skin. Studies have shown an increased number of inflammatory cells present in forearm skin of neuropathic individuals [155, 156], but not non-neuropathic ones [89].

During normal wound healing, macrophages shift from a pro-inflammatory or "M1" phenotype to a healing-associated or "M2" phenotype [67, 68] and express pro-inflammatory and anti-inflammatory genes, respectively [69, 70]. Indeed, relative expressions of pro-inflammatory and anti-inflammatory genes have been shown to be different in healing versus nonhealing human chronic DFUs [157]. Macrophages exhibited a prolonged pro-inflammatory response with high expression levels of pro-inflammatory molecules such as TNF-α, IL-1β, and MMP-9 during diabetic impaired healing [67, 158]. Macrophage dysfunction contributed to the defective healing seen in wounds of diabetic humans and mice [158]. In other studies, Nod-like receptor protein (NLRP)-3 inflammasome contributed to the sustained inflammatory state displayed by macrophages and is in part mediated by IL-1β in diabetic human and mouse wounds [158, 159]. Prolonged production of IL-1β has also been reported to reduce peroxisome proliferator-activated receptor γ (PPARγ) expression in diabetic wounds contributing to impaired healing [67].

The inflammatory response is further exacerbated and prolonged due to polymicrobial infection of the wound, usually with biofilm-forming bacteria that together sustain the influx of pro-inflammatory cells and at the same time impede host response to infection [160–162]. Infiltrating inflammatory cells such as leukocytes and neutrophils express ROS, that affect signaling pathways leading to the activation of transcription factors that control the expression of pro-inflammatory chemokines and cytokines as well as proteolytic enzymes and serine proteases [163, 164] and can cause damage to the ECM protein and impair fibroblast and keratinocyte function [165, 166].

The nuclear factor like 2 (Nrf2)-mediated oxidative stress response pathway plays a role in protecting cells against oxidative damage and promoting detoxification [167]. Nrf2 contributes to acute wound repair by regulating inflammation and promoting survival of keratinocytes under stress conditions [168]. Decreased levels of Nrf2 is associated with increased oxidized proteins and high glucose induces intracellular ROS in diabetic patients [169, 170] indicating an important role for Nrf2 in diabetic wound healing. Furthermore, in a streptozotocin-induced diabetic murine model, Nrf2 knockout mice exhibited a delay in wound healing compared with $Nrf2^{+/+}$ mice, partly as a result of higher oxidative DNA damage, increased MMP9 expression and apoptosis, and low TGF-β1 expression levels [171].

Infected DFUs are responsible for around 60% of lower-leg amputations [172, 173]. Local infection triggers the activation of neutrophils and causes the release of neutrophil extracellular traps (NETs). NETs are chromatin structures associated with antimicrobial molecules that serve to remove dead cells and infectious microorganisms [59, 60]. Neutrophils die by NETosis once infection is controlled [62]. However, deregulated NETosis can lead to tissue damage and excessive inflammation [174, 175]. High glucose levels and hyperglycemia are shown to increase NETs release and circulating markers of NETosis in diabetic patients [176, 177]. Furthermore, NETosis has been shown to delay diabetic wound healing in humans and murine models [178].

DFU Microbiome

A microbiome can be defined as the entirety of all microbes, their interactions, and their genomes within a particular environment. Commensal, symbiotic, and pathogenic microorganisms together make up the human skin microbiome. Because the skin functions as a barrier for the body, it is in direct contact with the outside world resulting in a combination of microflora that changes dynamically in a spatiotemporal manner [179, 180]. Injury to the skin disrupts this barrier, which leads to microbial influx and colonization of the wound, which may affect the healing process [181, 182]. Chronic wounds frequently have high polymicrobial burden prone to forming biofilms that are most commonly composed of *Staphylococcus*, *Pseudomonas*, and *Corynebacterium* [181, 183, 184]. The microbiome of DFUs has recently been recognized to play a significant role in

these hard-to-heal wounds [181] and is a potentially disease-modifying target for therapy.

While the microbial diversity of healthy human skin is already appreciated [179], it is necessary to better understand microbial inhabitants and pathogens and the role they play in the pathophysiology of impaired wound healing such as in DFUs. It is important to note that microorganisms can vary greatly between individuals and between different sites on an individual's skin [179], which is influenced by genetic and environmental factors [185]. New high-throughput sequencing technologies using 16S rRNA gene-based analyses provide a better resolution and surpass the limitations of traditional culture methods and have greatly expanded our understanding of the human microbiome [181, 186, 187]. There is an inherent bias in culturing microbes, as only the microbes suited for the chosen media can flourish. Additionally, microbes grow at varying rates and compete with each other on this media [181], which can skew the results. High-throughput sequencing of 16S rRNA genes provides much faster results and is less vulnerable to errors due to contamination, microbial viability, or sampling technique. The major limitation of this approach is that it does not differentiate between viable and nonviable bacteria [181]. This technology can be used to generate wound microbiome "footprints" that together with improving computational analyses and rapidly expanding reference databases can enhance our understanding of chronic wounds, especially DFUs and ultimately guide clinical decision-making [181, 187].

A recent study by Wolcott et al. explored the microbiomes of 2963 patients with chronic wounds including DFUs, VLUs, decubitus ulcers, and nonhealing surgical wounds [188]. While this study used a heterogeneous population and did not associate microbiomes with clinical factors, it is a large step forward in our understanding of the microbial "footprints" of chronic wounds. Interestingly, this study found that the microbiomes of these chronic wounds did not differ significantly by wound type or patient demographics [188], suggesting a common link in their pathogenesis with regard to microbiota. The most prevalent microbes in this large sample of chronic wounds were *Staphylococcus* followed by *Pseudomonas* [188]. Notably, many of these wounds had large numbers of anaerobic bacteria as well [188].

Taking a step further, a recent study by Gardner et al. specifically analyzed the DFU microbiome of 52 patients and found that variations in microbial diversity and amount were associated with significantly different clinical outcomes [187]. Using the 16S rRNA gene PCR method the investigators determined that culture-based methods underestimated the bioburden and biodiversity of the DFU microbiome [187]. For example, 40% wound cultures grew *Staphylococcus*, 27% anaerobes, and 35% Proteobacteria, while 16S rRNA PCR identified *Staphylococcus* in 94% of DFUs and both anaerobes and Proteobacteria in 100% of these wounds [187]. Additionally, this study found that deep ulcers and those of longer duration have a more diverse microbiome with higher amounts of anaerobes and Proteobacteria [187], while superficial ulcers and those of shorter duration have a higher relative abundance of *Staphylococcus* [187]. However, this contrasts to a study of the microbiome in DFUs complicated by osteomyelitis in which the ulcers were unquestionably very deep and *Staphylococcus* was identified in 100% of them identified [160].

In short, the microbiome of DFUs certainly has translational potential, but it should be appreciated that many other factors can influence this intricate network of microbes and its potential correlation to clinical outcomes. More longitudinal studies are needed before we can adequately correlate DFU microbiome to clinical outcomes. .

Stem and Progenitor Cells in Wound Healing

The ability of skin to replenish itself and contribute to tissue renewal and the overall wound healing process relies on resident epidermal stem cells. They are found in three distinct stem cell niches in the skin, including the basal layer of the epidermis (stratum basale), the base of sebaceous glands, and the "bulge region" of the hair follicle [189, 190]. The latter of these three has only been identified in mice, not humans. The microenvironments of these niches are important for modifying the activity and fate of the stem cells that reside in them [191]. Stem cells have been shown to mobilize and migrate to areas of wounded and ischemic skin tissue where they promote wound healing, reepithelialization, and angiogenesis [189, 192–194].

There are two proposed mechanisms by which stem cells maintain homeostasis of healthy epithelium. In the classic hierarchical model, stem cells in the basal layer give rise to transit amplifying daughter cells, which undergo a finite number of cell divisions as they travel upward before becoming terminally differentiated [189, 195, 196]. In this model, the stem cells and their progeny are organized in epidermal proliferative units. Recently, the stochastic model of homeostasis has emerged to challenge the classic model. In the stochastic model, epidermal stem cells can divide an unlimited number of times into two undifferentiated basal cells, two terminally differentiated cells, or one of each [189, 192, 197, 198]. Studies support both models, which is most likely explained by variations in epidermis at different anatomical sites [189, 198]. Resident stem cells are quiescent in healthy unwounded skin; however they lose their quiescence in response to injury and are recruited to replace the damaged tissue [199–201]. In addition to their role in proliferative renewal of the epidermis, there is new evidence that stem

cells have the ability to modulate and accelerate the wound healing process indirectly through paracrine signaling of growth factors and chemokines [51, 189]. In fact, mesenchymal stem cell (MSC) exosomes have been shown to induce proliferation and migration of fibroblasts in both acute and chronic wounds as well as promote angiogenesis [51]. Exosomes are small membrane-bound vesicles 30–120 nm in diameter secreted by many different cell types that contain transcription factors and genetic material and function in intercellular communication [51]. The doors are open to novel stem cell therapies and different delivery mechanisms with our new understanding of the exosome-mediated paracrine effects of stem cells.

Because of their diverse functions and major role in the wound healing process, stem cells are rapidly emerging as potential treatments for chronic wounds, especially DFUs. Stem cells in general have been used successfully to treat both acute and chronic wounds and have been shown to accelerate wound healing, facilitate reepithelialization, and promote angiogenesis [202, 203]. To date, many lines have been identified to have therapeutic potential including MSCs, bone marrow-derived mesenchymal stem cells (BM-MSCs), umbilical cord-derived MSCs, adipose-derived stem cells (ASCs), placenta-derived stem cells, bone marrow-derived mononuclear cells (BM-MNCs), and bone marrow-derived endothelial progenitor cells [51, 189, 193, 202, 204–206]. MSCs have received the most attention and are most commonly used in animal studies, preclinical, and clinical trials so far [189, 202]. Overview of the ongoing or completed clinical trials registered on www.clinicaltrials.gov is summarized in Table 7.3. It should be noted that this is a dynamic area of study, and this list is likely to change quickly.

Although invasive and costly, bone marrow aspiration is a reliable and quality source of progenitor cells. The process involves aspiration, centrifugation, and further maintenance of growth of the cells [193]. Because marrow is rich in progenitor cells, many investigators in the field of wound healing favor it, especially when using human subjects. In fact, there is evidence that autologous whole bone marrow, as opposed to isolated lines of various stem cells, has the greatest positive effect on wound healing in vitro and in vivo [207]. Others have demonstrated improved healing in DFUs

Table 7.3 Clinical trials of therapy with stem cells for diabetic foot ulcers as of August 2016

Conditions	Intervention	Study phase	ClinicalTrials.gov Identifier
DFU, critical limb ischemia	Umbilical cord mesenchymal stem cells	Phase I Phase II	NCT01216865
Peripheral vascular disease, ischemia, DFU	Adipose-derived stem cell	Phase I	NCT02831075
Peripheral vascular disease, ischemia, DFU	Umbilical cord mesenchymal stem cells	Phase I	NCT02834858
Peripheral vascular disease, ischemia, DFU	Mesenchymal stem cells	Phase I	NCT02796079
DFU, lower limb ischemia	Autologous mesenchymal stem cells	Phase I	NCT02304588
Diabetic critical limb ischemia	Autologous bone marrow stem cells and tissue repair cells	Phase II	NCT01065337
DFU, critical limb ischemia, leg ulcers	Granulocyte colony stimulating mobilized autologous peripheral blood mononuclear cell	Phase I Phase II	NCT00922389
DFU	Allogeneic mesenchymal stem cells	Phase II	NCT02619877
DFU, venous ulcer, pressure ulcer	Adipose-derived stem cells	Phase II	NCT02092870
Critical limb ischemia	Autologous bone marrow stem cell	Phase II	NCT01232673
Type 1 and 2 diabetes mellitus with foot ulcers	Allogeneic bone marrow-derived mesenchymal stromal cells	Phase I Phase II	NCT01686139
DFU	Autologous endothelial progenitor cells	Not available	NCT02474381
Diabetes, critical limb ischemia	Vascular progenitor cells	Not available	NCT01269580
Wounds, DFU, burns	Medical collagen membrane with mesenchymal stem cells	Phase I Phase II	NCT02672280
DFU, critical limb ischemia	Autologous bone marrow mesenchymal stem cells and mononuclear cells	Phase I	NCT00955669
DFU, leg ulcer, ischemia	Autologous bone marrow cell concentrate	Phase II Phase III	NCT00434616
DFU	Allogenic adipose-derived mesenchymal stem cells in hydrogel sheet	Phase I	NCT02394886
Lower extremity ischemia, leg ulcer, DFU	Autologous bone marrow-derived mononuclear cells	Phase I Phase II	NCT01903044
DFU	Autologous bone marrow mononuclear cells	Phase I Phase II	NCT00872326
DFU	Intra-arterial infusion of autologous bone marrow cells	Phase I Phase II	NCT00987363

using total nucleated bone marrow cells [208]. Autologous transplantation of isolated lines of either BM-MSCs or BM-MNCs has consistently been shown to improve wound healing rates and epithelialization [209, 210]. Additionally, bone marrow progenitor cells have been shown to improve peripheral circulation and boost angiogenesis in humans to support wound healing [210]. There is some evidence that BM-MSCs improve wound healing rates greater than BM-MNCs [211], but again, both types of marrow progenitor cells are showing efficacy when added to standard of care therapy. Therefore, bone marrow is a rich source of progenitor cells and other components with the potential to enhance wound healing.

Hematopoietic stem cells are another family of progenitor cells gaining attention for their potential application to chronic wound healing and DFUs, especially their potential to improve circulation in diabetic limbs. $CD34^+$ endothelial progenitor cells are the most abundant and thus are the most frequently studied. Additionally, there is new evidence that $CD34^+$ cells are lacking or decreased in nonhealing ulcers [212], which further supports the exploration of these progenitor cells in DFUs and their therapeutic potential. These cells can be isolated from bone marrow or peripheral blood after administration of cytokines like granulocyte macrophage colony stimulating factor (GM-CSF) [193]. So far, animal studies have been promising, but there have been no major trials yet in humans. A small pilot study with five patients in Japan demonstrated the safety and feasibility of $CD34^+$ endothelial progenitor cells in patients with nonhealing DFUs [213], but larger multicenter studies are needed before their efficacy in chronic diabetic wounds can be properly assessed.

Adipose tissue is emerging as another source progenitor cells. ASCs are enticing because they are easily obtained through liposuction procedures, and adipose tissue is more abundant than marrow or other sources [193]. There is growing evidence of their efficacy in animal models [193], but there is a paucity of human trials at this point. The major limitation for ASCs is that it is very difficult to degrade the surrounding tissue to isolate the stromal vascular fraction or the ASCs contained therein.

Human amnion and chorion membranes have also come forth with potential to improve healing in chronic lower extremity wounds. Partly, interest has arisen because fetal skin wounds repair rapidly and without any scar formation, although the exact mechanisms are not fully understood. These membranes have been found to promote tissue regeneration, wound healing, and even recruiting resident stem cells into wounded areas [214]. Recently, a randomized controlled trial showed that a placental membrane with growth factors, MSCs, fibroblasts, and epithelial cells showed significantly improved healing and less complications than standard of care therapy in human DFUs [215]. Although this does not prove efficacy for placental membranes or MSCs individually, it further supports the concept of using combined approaches when managing these difficult-to-heal wounds. In short, placental products and membranes have the potential to improve treatment of chronic wounds by improving wound healing themselves and by supporting transplanted cells.

Lastly, recent developments allow for the reprogramming of differentiated somatic cells into induced pluripotent stem cells (iPSCs) [1, 189, 216]. Many different cell lines can serve as a source of iPSCs in humans including keratinocytes, fibroblasts, lymphocytes, and liver cells [189], which can be reprogrammed via retroviral transduction [216, 217]. iPSCs have been shown to differentiate into many cell types including keratinocytes and fibroblasts, which have been used to create human skin equivalents [1]. Interestingly, iPSCs have also been reprogrammed from DFU fibroblasts [216, 218], which highlights their regenerative potential in these wounds. Their advantages include that they can be derived from autologous cells to circumvent rejection, can produce a multitude of differentiated cells necessary for wound healing, and can be reprogrammed into specifically desired cell types. Disadvantages include cancer risk due to retroviral vectors, inefficient reprogramming resulting in low iPSC yield, genetic instability, and potential immunogenicity [219]. However, newer techniques for safer reprogramming are in development. Thus, iPSCs are an intriguing source of progenitor cells with the potential to improve DFU wound healing in the future.

Stem cells and progenitor cells can be delivered to wounds locally (e.g., sprays or injections) or systemically [189, 193]. Systemic administration carries the added risk of cell trafficking and malignancy as well as difficulty targeting the cells to the wound [193]. Direct application of stem cells has been hindered by low cell proliferation and survival rates with a lack of persistence in the wounds [220]. Thus, there is a strong need for alternative strategies to optimize cellular therapy. So far, skin scaffolds and dermal matrices have been developed to enhance cell survival. They can be classified as natural, synthetic, or hybrid, and they promote cell proliferation and regeneration by providing a spatiotemporal environment [189, 221]. Examples of these include the successful direct application of autologous MSCs with a fibrin spray system in both acute and chronic wounds in humans and mice [222], application of autologous BM-MSCs embedded in collagen matrices [223], and delivery of ASCs within an acellular cadaveric dermal matrix [206]. Novel matrix design methods such as electrospinning and 3D bioprinting are also under investigation for their application to optimizing cell survival.

In summary, there is tremendous interest and profound therapeutic potential for stem cells and progenitor cells in the field of chronic wounds and DFUs (Table 7.3). They have

shown promise in speeding reepithelialization, angiogenesis, and improving the overall wounding healing process. Although showing great promise, it is important to remember that no stem cell therapy to date has accumulated enough evidence to earn FDA approval for treatment of chronic wounds. There are certain limitations for each stem cell line and each delivery method. Because the pathogenesis of DFUs is so complex, it is likely that future treatments for these hard-to-treat wounds will involve a multimodal approach utilizing stem cells along with other local and systemic therapies.

Treatment for DFUs

Standard treatment for all DFUs includes glycemic control, debridement of necrotic tissue, control of infection, use of moist compressive dressings, offloading to protect from pressure or trauma related to ambulation, and adjuvant hyperbaric oxygen therapy (HBO) in select patients [224, 225]. In the case of clinically significant arterial insufficiency, revascularization by surgical bypass or endovascular techniques is required for delivery of essential oxygen and nutrients to support the healing process [224, 226]. However, the ability to create clinically significant improvements in wound healing after revascularization is limited because vascular procedures can only be performed at the level of large- and medium-sized arteries, while many diabetes-related complications are due to compromise of the microvascular circulation [53, 226]. Currently, there are only four FDA-approved therapies for DFUs (Table 7.4) including two dermal substitutes (Dermagraft, Integra omnigraft), one human skin equivalent (Apligraf), and recombinant human platelet-derived growth factor (rhPDGF) [47, 48, 227, 228].

Table 7.4 Current FDA-approved DFU therapies

Treatment	Description	References
Becaplermin	Recombinant human platelet-derived growth factor (rhPDGF) for use in neuropathic DFUs with adequate blood supply	Wieman et al. (1998) [258] Smiell et al. (1999) [228]
Apligraf	Human skin equivalent composed of a dermal layer containing human fibroblasts and connective tissue and an epidermal layer consisting of keratinocytes	Falanga et al. (1999) [259] Veves et al. (2001) [47]
Dermagraft	Human dermal substitute consisting of cryopreserved human fibroblasts, extracellular matrix, and a bioabsorbable scaffold	Marston et al. (2003) [48]
Integra Omnigraft	Dermal matrix substitute composed of silicone in the top layer and bovine collagen and shark chondroitin on the bottom layer	Driver et al. (2015) [227]

Offloading is a treatment modality that redistributes pressure away from the DFU area to facilitate more effective wound healing. This can be achieved by orthotics, braces, casts, and wound care dressings [229, 230]. A recent systematic review of offloading in DFUs found that it is key to improve wound healing and that total contact casts were the most effective at achieving ulcer healing [231]. While offloading has been recognized as a standard of care for DFUs, new devices and techniques are frequently under investigation and in development [225].

Surgical debridement of necrotic tissue surrounding DFUs has been a standard of therapy for many years. Debridement promotes wound healing by removing nonviable necrotic tissue, which is detrimental to the wound healing process [232]. By removing hyperkeratotic epidermis (callus), necrotic dermal tissue, debris, and bacteria, a chronic nonhealing wound can be converted to an acute wound environment that is better able to heal and respond to topical treatments. Although debridement is practiced ubiquitously in the management of DFUs as standard of therapy, and the rationale behind it is convincing, high-quality scientific evidence to support its role is lacking [232, 233]. Furthermore, debridement may work synergistically with other treatment modalities such as cell therapy and growth factors.

Growth factors are biological therapies for DFUs and may be useful in combination with surgical debridement. Becaplermin is an rhPDGF that has been shown to promote wound healing and is FDA approved for treatment of DFUs [10, 228, 234] (Table 7.4). PDGF aids the initiation of the inflammatory phase of wound healing by stimulating chemotaxis of neutrophils, macrophages, fibroblasts, and smooth muscle cells to the wound site [10]. Because of its cost, it is a reasonable choice in wounds that have not healed with more conservative treatment. Recombinant human epidermal growth factor (rhEGF) has also been shown recently to improve wound healing in DFUs [235, 236]. Recombinant human vascular endothelial growth factor (rhVEGF) has also been investigated for its application to DFU wound healing. It is thought to improve angiogenesis in diabetic ischemic limbs [237]. However, there are very few studies with high-quality evidence to support its use in DFUs, although a Phase II trial has recently completed (NCT00351767). rhVEGF may be limited because it promotes formation of disorganized blood vessels with sustained vascular leakage [10]. Moving to hematopoietic growth factors, G-CSF has been shown to promote wound healing in infected DFUs [10, 238], but current studies are small. It is thought to work by improving the defective neutrophil response that is characteristic for DFUs. Lastly, GM-CSF has shown efficacy in healing chronic venous ulcers [239, 240], but there is a lack of studies in DFUs to date. It works by promoting myofibroblast differentiation, wound contracture, recruitment of inflammatory cells, and facilitation of epidermal

proliferation [10]. Although initially considered as highly promising, growth factor therapy may have limited efficacy due to the highly proteolytic chronic wound environment and the lack of corresponding growth factor receptors [100, 116] required for the successful biological effect of these growth factors.

Cell therapy has been quite successful in the treatment of DFUs, with one human skin equivalent and two dermal substitutes garnering FDA approval for their application in these hard-to-heal wounds (Table 7.4). Graftskin (Apligraf) is a human skin equivalent composed of a dermal layer containing human fibroblasts and connective tissue without any blood vessels, hair follicles, or sweat glands and the epidermal layer consisting of keratinocytes [47]. There is high-quality evidence that this human skin bilayer accelerates DFU healing [47]. Similarly, Dermagraft is a human dermal substitute consisting of cryopreserved human fibroblasts, ECM, and a bioabsorbable scaffold that has demonstrated significantly improved healing in DFUs [48]. Much more recently, the Integra omnigraft has gained FDA approval for the treatment of DFUs [227] (Table 7.4). It is composed of silicone in the top layer and bovine collagen and shark chondroitin on the bottom layer [227]. Although it does not contain any human cells, it allows for immediate wound closure, dermal regeneration, and acts as a scaffold to facilitate autologous cell migration and proliferation to augment the healing process [227]. In all, cell therapies have seen the greatest advances so far in the treatment of recalcitrant DFUs.

For many years, skin grafting has been a successful treatment modality for burn wounds, especially wounds covering a significant portion of the total body surface area. Similar skin grafting techniques are now being explored in the treatment of nonhealing chronic wounds including DFUs. Cultured keratinocytes, otherwise known as cultured epithelial autografts (CEA), have been used to treat mostly acute full-thickness wounds. This method entails culturing a patient's own keratinocytes in vitro and allows for a large expansion ratio [241, 242]. This method has seen only minimal investigation with some evidence of efficacy in DFUs [243]. The traditional skin graft is known as a split-thickness skin graft that contains both epidermal and dermal layers. This graft can be taken from the donor site and directly transplanted onto the wound site or it can first be processed by a meshing device that allows for its expansion up to ten times its original surface area via the Meek method [244]. The orientation of the split-thickness graft is important as it must be grafted with the dermis side facing down into the wound [242, 245, 246]. While used mostly for acute wounds and burns, it has shown some efficacy in DFUs, but only in very small studies and case reports [247–249].

Recent advances in skin grafting techniques have potential for application to DFUs. Skin micrografts are made from minced split-thickness skin samples into 0.8 × 0.8 mm pieces, which allows for 100-fold expansion [242, 246]. Micrografting provides several advantages over traditional skin grafting techniques. First, the 100-fold expansion ratio allows for much greater wound area coverage from any given donor site. Second, it has been proven that the orientation of micrografts (dermal side up or down) does not impact efficacy or final appearance of the wound reducing associated time and labor [242, 245, 246]. Micrografting has shown efficacy in diabetic animal wound models [246], but no major clinical trials have been completed in humans yet. Along the same lines, pixel grafting is an emerging technique utilizing even smaller 0.3 × 0.3 mm skin grafts with an even greater expansion ratio [245]. Because pixel grafting is such a novel technique, there is a paucity of studies exploring its efficacy in chronic wounds, which currently limits its translation into clinical practice.

Various wound dressings can be useful in the management of DFUs. It is known that a moist wound environment provides the optimal conditions for cells involved in wound healing as well as promotes disposal of dead cells by the body [250], and thus moist dressings are preferred in the treatment of DFUs. A recent overview of systematic reviews on the topic of wound dressings assessed the evidence behind various dressings including absorbent dressings, alginate, hydrogel, permeable films, membrane dressings, soft polymers, foam, iodine-impregnated, and silver-impregnated dressings and found that there is currently no high-quality evidence favoring one type of dressing over another in DFUs [250]. It is important to consider the pros and cons of each as well as patient preference, compliance, and cost when choosing a dressing for their DFU.

HBO is a useful adjunctive therapy in DFUs with compromised microvasculature. Tissue level hyperoxia from HBO works synergistically with chemokines produced by keratinocytes and fibroblasts to recruit endothelial progenitor cells from circulation to the site of injury [3], which can improve blood flow and overall wound healing. Thus, success of HBO is dependent on sufficient production of chemokines by ulcer tissue [251]. Several RCTs have shown that HBO is efficacious as an adjunctive therapy in DFUs specifically [252, 253]. A potential limitation of HBO is increased oxidative stress in local ulcer tissue with prolonged use [253].

In all, there are a great variety of treatments available for DFUs. While there are many therapeutic options, each has their pros and cons that must be weighed when making a clinical decision. Because the pathogenesis of DFUs is so complex and many therapies work synergistically, the best treatment for any given DFU is likely a multimodal approach incorporating the standard of care plus newer adjunctive treatments and/or cell therapy depending on the patient. In spite of recent advances, the most effective treatment regimen has yet to be determined, and further research in this area is needed to optimally manage this devastating complication of diabetes.

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8 Neuropeptides, Inflammation, and Diabetic Wound Healing: Lessons from Experimental Models and Human Subjects

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Abstract

Diabetic peripheral neuropathy and vascular disease, along with trauma, have long been recognized as major risk factors for the development of diabetic foot ulcerations (DFUs). More recently, chronic inflammation, abnormal extracellular matrix (ECM) remodeling, and reduced wound neovascularization, as a result of dysregulated cell function with imbalanced secretion of cytokines, matrix metalloproteinases, and growth factors, have been implicated in DFU failure to heal. Therefore, researchers are now focusing their efforts on further understanding the cellular and molecular mechanisms of diabetes-associated impaired wound healing, in an attempt to identify new targets and novel potential therapeutic approaches for DFUs, which remain a serious unmet clinical need. A growing body of evidence suggests an important role of neuropeptides in skin repair, particularly in diabetes, where neuropeptide levels are diminished. On the other hand, there is emerging interest in dissecting the mechanisms of dysregulated inflammation, namely the changes in immune cells, such as macrophages and mast cells (MCs), in diabetic wound healing. Studies using in vitro and in vivo models of diabetic wound healing have considerably improved our understanding of the healing process. However, the currently available models have major caveats and are not ideal to study chronic, complicated, and multifactorial wounds, such as DFUs. In this chapter we summarize the involvement of neuropeptides and mast cells in diabetic wound healing, highlighting the most recent findings. We also discuss the benefits and limitations of the current wound healing models, emphasizing the need for confirmation and/or validation in multiple models and/or tissue specimens from human subjects.

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Neuropeptides and Diabetic Wound Healing

It has been estimated that up to 85% of DFUs are associated with diabetic peripheral neuropathy [1]. Diabetic peripheral neuropathy is associated not only with loss of pain sensitivity, especially at the lower extremities, rendering diabetic patients prone to disregard trauma in such areas, but also with reduced levels of neuropeptides. Neuropeptides are secreted by the small nerve fibers, both sensory and autonomic, as well as by dermal and epidermal cells [2, 3]. They not only relay information such as pain signals to the central nervous system, but participate in the inflammatory and proliferative phases of wound healing by binding to specific receptors that are found in various skin cells, including immune cells such as mast cells (MCs), as well as endothelial cells, fibroblasts, and keratinocytes [4]. In fact, neuropeptides can regulate the release of numerous cytokines and growth factors that are pivotal for wound repair and imbalanced in diabetes, including IL-1, IL-6, IL-8, TNF-α, and VEGF [2]. As a result, there is growing interest in the potential role of neuropeptides, namely Substance P (SP), neuropeptide Y (NPY), neurotensin (NT), calcitonin gene-related peptide (CGRP), and alpha-melanocyte-stimulating hormone (α-MSH), in diabetic wound healing [2, 3, 5]. As key players in the bidirectional neuro-immune/neuro-inflammatory axis [2, 4], it is anticipated that neuropeptides are involved in the healing of DFUs, where loss of sensory nerves, ineffective immune and inflammatory responses,

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and dysregulated inflammation are present. While focusing mostly on Substance P (SP), our group has also investigated the roles of neuropeptide Y (NPY) and neurotensin (NT) in diabetic wound healing.

Substance P and Diabetic Wound Healing

Substance P (SP) is an 11 amino acid peptide that belongs to the tachykinin neuropeptide family, encoded by the *TAC1* gene. SP exerts its actions by activating three primary types of neurokinin (NK) receptors—NK1R, NK2R, and NK3R, with NK1R being the predominant and with highest affinity—, and is degraded by the enzyme neutral endopeptidase (NEP).

For decades, reports have suggested and/or demonstrated that SP participates in acute noncomplicated wound healing. SP is known to cause vasodilation [6, 7], to stimulate proliferation and migration of endothelial cells [8, 9], fibroblasts [10, 11], and keratinocytes [12], as well as to recruit and activate immune cells [13–15]. Besides its trophic and chemoattractant effects, SP has also proven to be pro-angiogenic in vitro and in vivo [16–18]. Based on such properties, and on the fact that SP is promptly released following cutaneous injury, it was predicted to improve wound healing. In fact, SP treatment was shown to ameliorate acute wound healing in rodents [19]. Of interest, studies have reported reduced SP expression in skin biopsies from diabetic subjects [20]. Therefore, emerging studies are starting to explore the involvement of SP in healing of diabetic wounds—in diabetic corneal wounds [21, 22], where epithelial cells are the major effectors, and also in diabetic cutaneous wounds [3, 23], which involve a complex interplay between dermal and epidermal cells, and are characterized by a chronic inflammatory and highly proteolytic environment.

Studies by our group have shown reduced gene and protein expression of SP, as well as reduced gene expression of the main SP receptor—neurokinin-1 receptor (NK1R)—, in the unwounded skin of diabetic rabbits when compared to their nondiabetic counterparts [24, 25]. This reduced SP skin expression was accompanied by a local chronic inflammatory state, indicated by an increased baseline pro-inflammatory cytokine expression without further increase in response to wounding, and resulted in delayed wound closure [6].

In acute noncomplicated healing, M1-activated macrophages are predominant during the inflammatory phase, as they initiate an acute inflammatory response to injury, while during the proliferative phase M2 macrophages take over to promote angiogenesis and granulation tissue formation [25–28]. However, diabetic rabbits showed an elevated baseline skin M1/M2 macrophage ratio that persisted until the later stages of wound healing (10 days post-wounding), suggesting a chronic pro-inflammatory environment.

In agreement with our diabetic rabbit ear model findings, we have shown reduced SP expression and increased NEP expression in the skin of diabetic mice (Fig. 8.1) [29]. Importantly, in diabetic human subjects, the circulating levels of SP were reduced, the skin gene expression of SP was reduced, and both the skin gene and protein expression of NEP were increased when compared to healthy control subjects, suggesting that SP bioavailability is severely decreased in diabetes. In addition, skin gene expression of the SP receptor NK1R was reduced in diabetes. Similarly to the diabetic rabbit, the diabetic murine wound healing model was characterized by increased pro-inflammatory cytokine expression and elevated M1/M2 macrophage ratio at baseline, lack of a robust acute inflammatory response at the earlier stages of healing (day 3 post-wounding), and defective inflammation resolution at the later stages (day 10 post-wounding), with failure to switch from the pro-inflammatory M1 to the pro-regenerative M2 phenotype, and delayed wound closure [29]. Moreover, genetically modified mice deficient in SP and related tachykinins (TAC1KO mice) or in the SP receptor NK1R (NK1RKO mice) also had delayed wound closure compared to their wild-type (WT) controls and presented chronic low-grade inflammation, with elevated baseline skin expression of pro-inflammatory markers and elevated M1/M2 ratio, similar to the diabetic animals.

Notably, the presence of diabetes did not further delay wound healing in NK1R-deficient (NK1RKO) mice [29]. Furthermore, using the rabbit sham/ischemic/neuroischemic ear model, our group has shown that diabetes impairs wound healing in both sham and ischemic conditions, but does not have an additional negative impact on the healing of neuroischemic wounds. Together, these results suggest loss of neuropeptide (in particular SP) function as an important component of diabetes-associated impaired wound healing. Interestingly, neuroischemic wounds, which showed the most delayed healing, had the highest baseline skin macrophage infiltration, and the highest wound M1/M2 ratio at later stages of healing (day 10 post-wounding) [25].

Importantly, we have demonstrated that topical application of SP to the wounds of both diabetic mouse dorsum and diabetic neuroischemic rabbit ear accelerates closure and ameliorates healing (Fig. 8.2) [29]. SP topical treatment induced the expression of pro-inflammatory cytokines, such as MCP-1, IL-6, and KC (mouse homolog of human IL-8), and increased the number of M1 macrophages during the early stages (inflammatory phase) of healing, whereas it reduced pro-inflammatory cytokine expression and polarized macrophages to the M2 phenotype during the later stages,

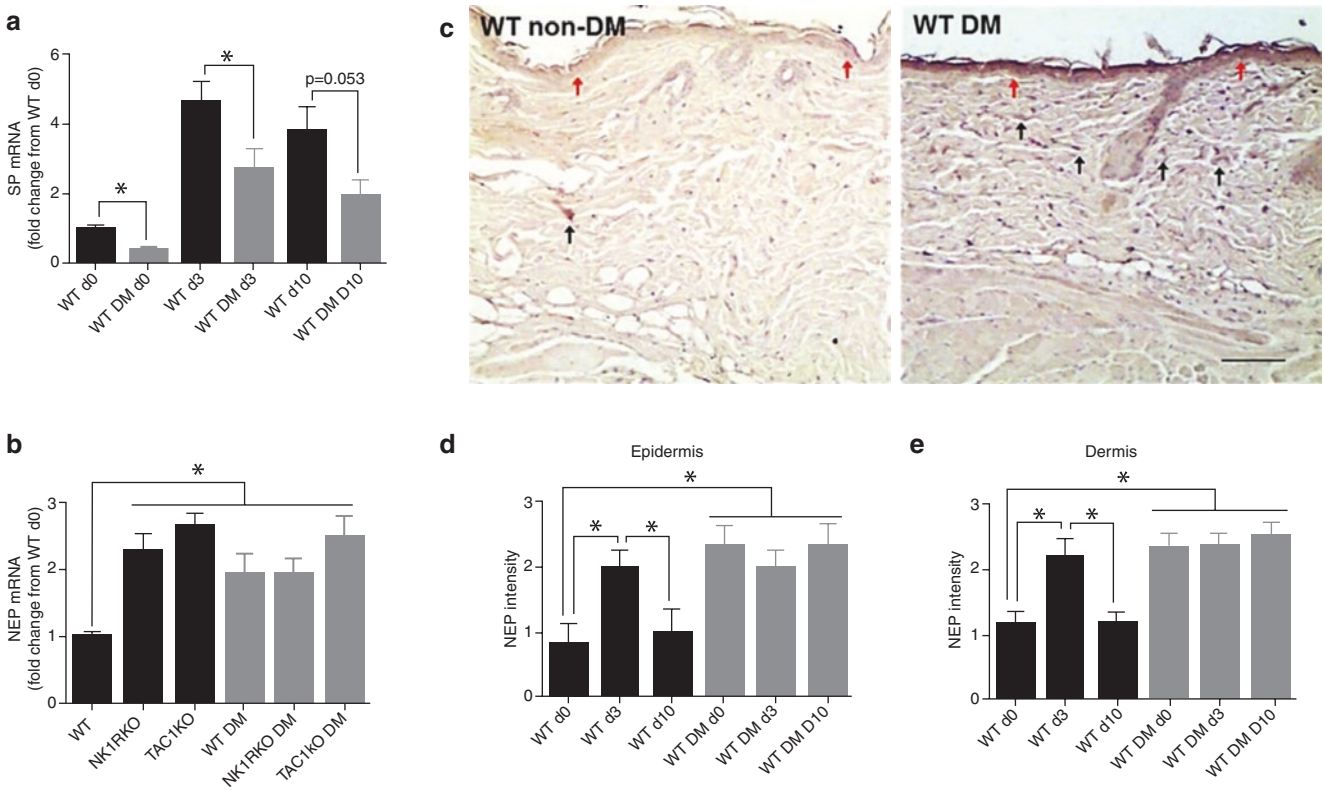

Fig. 8.1 Substance P (SP) skin expression is reduced and neutral endopeptidase (NEP) skin expression is increased in diabetic mice. (**a**) Skin gene expression of SP was reduced in wild-type mice with diabetes mellitus (WT DM) compared to their nondiabetic wild-type controls (WT). (**b**) Skin gene expression of NEP was increased in WT DM mice, as well as in neurokinin 1 receptor knockout (NK1RKO) and knockout (TAC1KO) mice at baseline (Day 0, d0). (**c**) Representative images of NEP staining in baseline (d0) skin from WT non-DM and WT DM mice. DM mice have a higher NEP intensity in the epidermis (**red arrows**) and a higher number of NEP-positive cells in dermis (**black arrows**) compared with WT non-DM mice at day 0. Scale bar: 100 μm. NEP staining intensity was increased in the (**d**) epidermis and (**e**) dermis of WT DM mice at baseline (d0). In WT non-DM mice, NEP intensity increased at d3 post-wounding, but returned to baseline levels by d10, whereas in WT DM mice it remained elevated throughout the healing process. Data represent the mean ± SEM. *$p < 0.05$. Copyright© Elsevier. Adapted from Leal, Carvalho, Tellechea et al., Substance P promotes wound healing in diabetes by modulating inflammation and macrophage phenotype, American Journal of Pathology 2015 Jun;185(6):1638–48 with permission from Elsevier

therefore allowing inflammation resolution and progression to the proliferative phase (Fig. 8.3).

In agreement with human studies that showed increased MMP-9 levels in the skin of diabetic subjects [30], as well as in diabetic and other chronic wounds [31–33], MMP-9 expression was increased in both unwounded skin and wounds of our mouse model of diabetic wound healing [29]. Of interest, TAC1KO and NK1RKO mice also presented increased baseline skin MMP-9 expression, whereas topical SP treatment reduced MMP-9 expression post-wounding in diabetic mice. This suggests that loss of SP function may contribute to chronic elevated expression of MMP-9 in diabetic skin and wounds, and that SP treatment may attenuate it.

More recently, other researchers have studied the effect of systemically administered SP on cutaneous wound healing in diabetic mice. As expected, their results confirmed that SP accelerates diabetic wound closure and prevents the prolonged inflammatory response to injury [34]. Interestingly, the effects of systemic SP observed in the serum of the diabetic mice were similar to the ones of topical SP in the wound tissue at the later stages of healing, namely elevated M2 monocytes in the peripheral blood mononuclear cell population/elevated M2 macrophages in the wound margins, and reduced TNF-α circulating levels/reduced TNF-α wound expression, respectively.

The ability of SP to induce the switch from M1 to M2 macrophages has also been confirmed in vitro [35, 36]. Of note, SP treatment promoted macrophage M2 polarization, and release of pro-inflammatory factors via activation of the NF-κB pathway in a coculture model of fibroblasts and resting macrophages [35], whereas it suppressed NF-κB activation and reduced the production of pro-inflammatory cytokines and enzymes in LPS-stimulated murine macrophages [36],

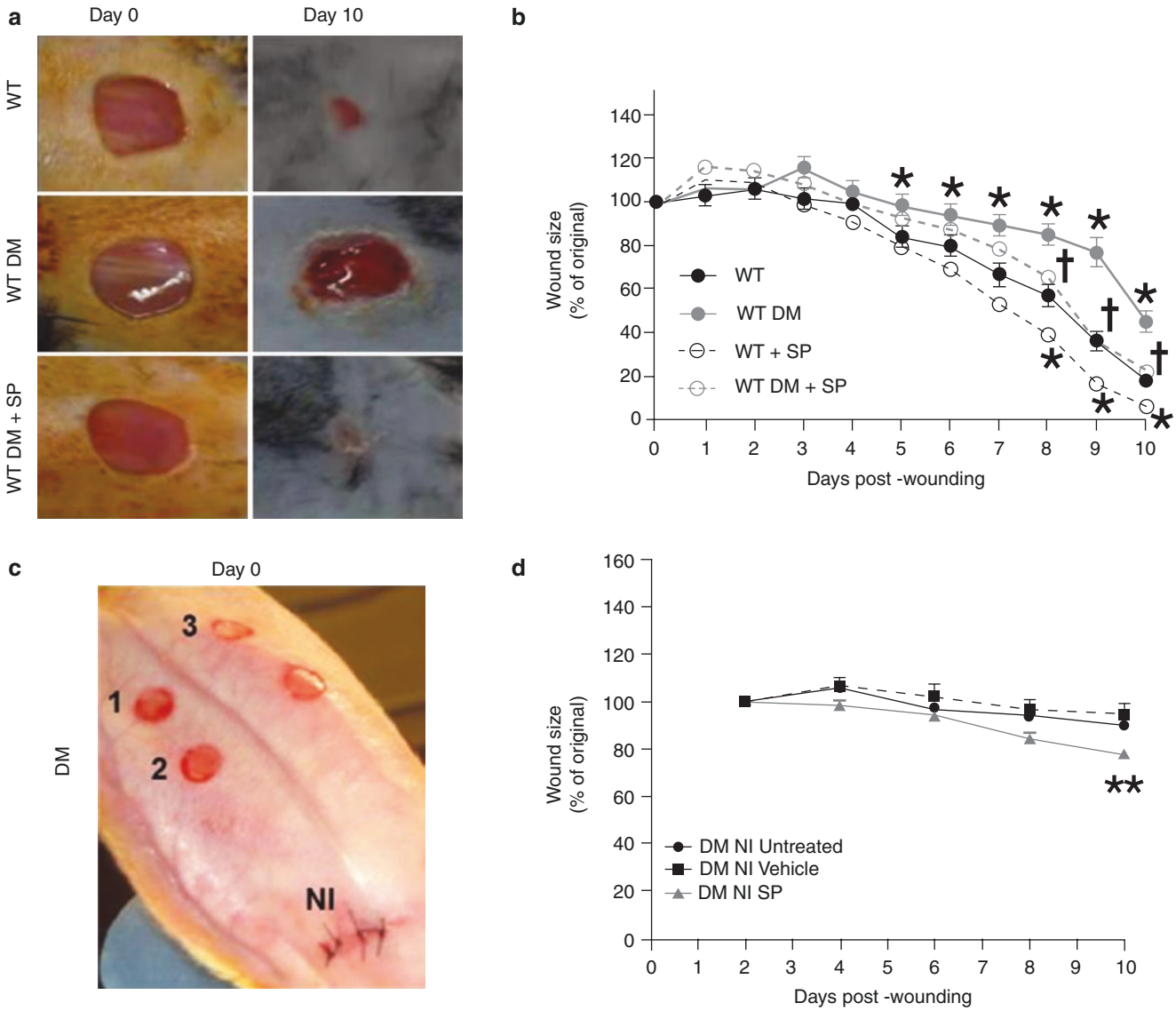

Fig. 8.2 Substance P (SP) topical treatment accelerates wound healing in mouse and rabbit models of diabetes. (**a**) Representative images of mouse dorsal skin wounds at baseline (Day 0) and Day 10 of wild-type nondiabetic (WT), WT diabetic (WT DM), and WT diabetic SP-treated wounds (WT DM + SP). (**b**) WT DM mice showed delayed healing compared to WT non-DM mice. Topical SP accelerated wound closure in both WT and WT DM mice. Data represent mean ± SEM.*$p < 0.05$, compared to WT. †$p < 0.05$, compared to DM. (**c**) Representative image of the neuroischemic rabbit ear wounds at Day 0. 1: untreated; 2: vehicle-treated; 3: SP-treated; NI: neuroischemia. (**d**) Topical SP improved wound healing in a DM NI rabbit wound healing model. Data represent mean ± SEM. **$p < 0.01$ compared to untreated and vehicle-treated wounds. Copyright© Elsevier. Adapted from Leal, Carvalho, Tellechea et al., Substance P promotes wound healing in diabetes by modulating inflammation and macrophage phenotype, American Journal of Pathology 2015 Jun;185(6):1638–48 with permission from Elsevier

again suggesting that it may act as pro- or anti-inflammatory depending on the environment/experimental conditions.

In summary, SP acts as a modulator of the inflammatory response to injury, and may be particularly important in the treatment of diabetic cutaneous wounds, where it appears to convert the chronic low-grade inflammation into an early acute inflammatory response followed by inflammation resolution with progression to the proliferative phase of healing. NEP inhibitors have been suggested as a potential treatment for DFUs, but their serious adverse effects, such as angioedema, impede its use [37, 38]. Alternatively, local delivery of SP via biomaterials that gradually release SP [39], protecting it from rapid degradation, or topical treatment with more resistant SP analogs, have the potential to promote wound healing in diabetes, without major off-target effects expected.

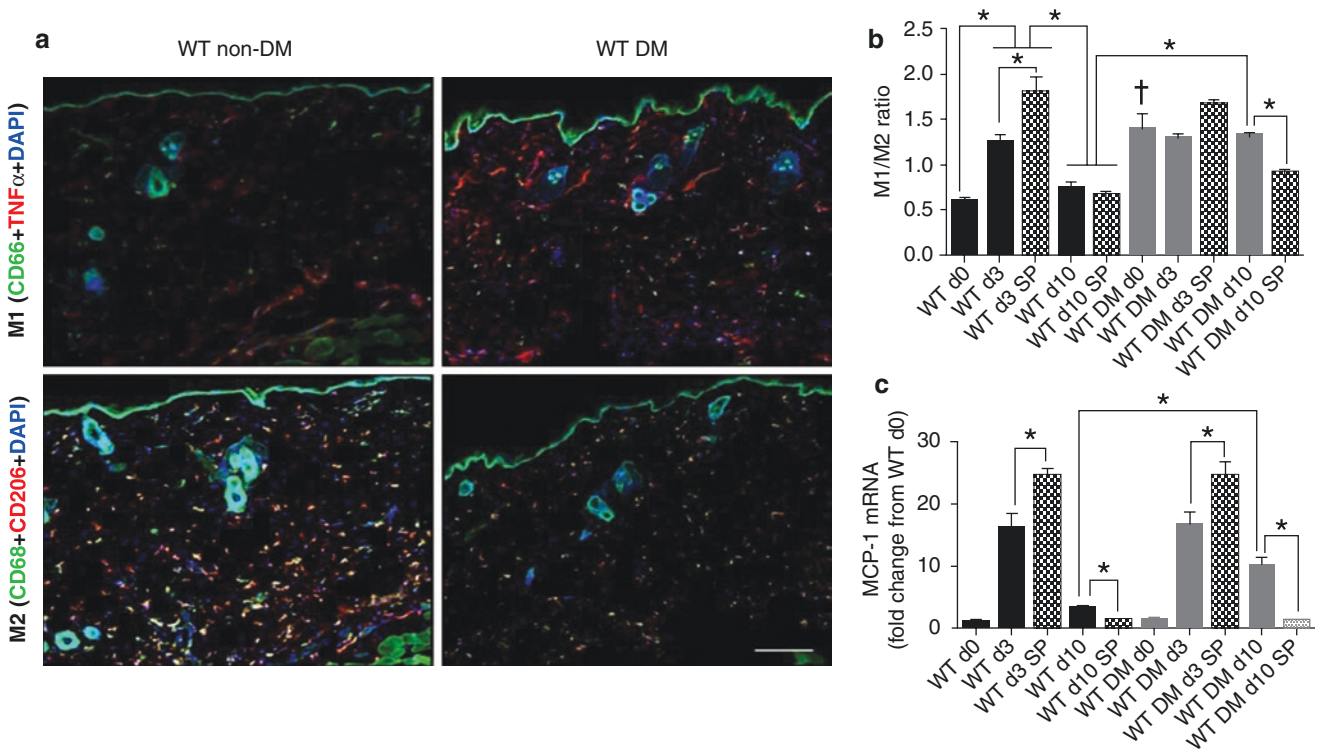

Fig. 8.3 SP modulates skin macrophage phenotype during healing. (**a**) Representative images of M1 and M2 macrophages in wild-type nondiabetic (WT non-DM) and diabetic (WT DM) mouse skin. Scale bar: 100 μm. M1 (upper panel) and M2 (lower panel) are denoted by the yellow-orange stain resulting from triple positive stain with CD68, TNF-α and DAPI (for M1) or CD68, CD206 (for M2) and DAPI. (**b**) M1/M2 ratio was higher in DM at day 0 (d0). In non-DM mice, M1/M2 peaked at day 3 (d3) and returned to baseline levels at day 10 (d10), while it was persistently elevated in DM mice. SP increased M1/M2 at d3 and reduced it at d10. (**c**) MCP-1 skin gene expression peaked at d3 and returned to pre-wounding levels at d10 in WT non-DM mice, but not remained elevated at d10 in DM mice. In both WT non-DM and DM mice, SP treatment further increased MCP-1 at d3 and reduced it at d10. Data represent mean ± SEM. *$p < 0.05$; †$p < 0.05$ compared to WT non-DM d0. Copyright© Elsevier. Adapted from Leal, Carvalho, Tellechea et al., Substance P promotes wound healing in diabetes by modulating inflammation and macrophage phenotype, American Journal of Pathology 2015 Jun;185(6):1638–48 with permission from Elsevier

Neuropeptide Y and Diabetic Wound Healing

The 36 amino acid peptide Neuropeptide Y (NPY) belongs to the pancreatic polypeptide family and is one of the most abundant neuropeptides in mammals [2]. NPY acts by binding to its G-protein coupled receptors—Y1, Y2, Y4, Y5, and Y6—and is widely distributed through the central and peripheral nervous system, but can also be found in other tissues, including the gastrointestinal tract and the skin [40–42].

Most studies on NPY are related to its neuroendocrine effects on the central nervous system, where NPY acts as a potent orexigenic peptide regulating appetite, energy metabolism, and body weight [43]. However, there is evidence that NPY also influences metabolic functions in peripheral tissues. NPY was shown to suppress lipolysis and promote adipogenesis [44, 45], suggesting beneficial effects on lipid uptake and storage in adipose tissue. Accordingly, mice deficient in NPY or in its Y1 receptors developed insulin resistance and adipose tissue inflammation following high fat diet feeding [46, 47]. Another study confirmed that NPY modulates obesity-induced inflammation, as loss of NPY expression from hematopoietic cells increased the number of adipose tissue macrophages and NPY receptor blockade induced dendritic cell maturation and secretion of IL-6 and TNF-α [48]. Nonetheless, in a model of combined chronic stress and diet-induced obesity, the expression of NPY and Y2 receptor were associated with insulin resistance and increased numbers of adipose tissue macrophages [49, 50].

Similarly to obesity, diabetes affects NPY levels, distribution, and action in a complex fashion. Whereas elevated circulating levels of NPY have been associated with type 2 diabetes [51], and recently NPY has been identified as a minor autoantigen in type 1 diabetes [52, 53], the skin expression of this neuropeptide is reduced in both type 1 and type 2 diabetes [54, 55].

NPY has been reported to participate in angiogenesis as well as in inflammatory and immune responses both in vitro and in vivo. Namely, NPY induces proliferation, migration, and tube formation of endothelial cells [56], promotes angiogenesis [57], and regulates immune responses including leukocyte trafficking, macrophage function, phagocytosis and cytokine release, as well as antigen presentation and antibody production [58–61], all of which are important in wound healing. The effects of NPY on immune cells are complex and, similarly to SP, NPY can induce either pro- or anti-inflammatory activities. For example, NPY activated macrophages in adult mice, but suppressed their chemotactic and phagocytic capacities in aged mice [62, 63]. Likewise, NPY induced nitric oxide (NO) release from LPS-stimulated peritoneal macrophages in young rats, but not from their older counterparts [64]. Such findings suggest that NPY may potentiate acute inflammatory responses while protecting against inflammation in chronic inflammatory conditions.

The role of NPY in healing has been mostly studied in ligament and tendon rupture [65–67], as well as in vascular remodeling [68–70], but recently researchers have begun to explore its involvement in cutaneous wound healing. Namely, mice deficient in the Y2 receptor have shown delayed wound healing and reduced skin neovascularization [71]. Studies by our group have demonstrated that, similarly to SP, the gene and protein expression of NPY is dysregulated in the diabetic rabbit ear model [24, 25]. In particular, baseline NPY skin protein expression was reduced in the diabetic animals, while its gene expression was reduced post-injury. In addition, compared to their nondiabetic counterparts, NPY gene expression was lower in diabetic ischemic and diabetic neuroischemic wounds [25]. Interestingly, no differences were observed in the gene expression of receptors Y2 and Y5, which are known for their pro-angiogenic effects. The above findings suggest that NPY participates in the healing of diabetic wounds. However, further investigation is needed to unravel the mechanistic pathways involved.

Neurotensin and Diabetic Wound Healing

Neurotensin (NT) is a 13 amino acid bioactive peptide primarily distributed in the central nervous system and in the gastrointestinal tract [72, 73]. NT mediates its functions through the binding to two G-protein coupled receptors—neurotensin receptor 1 (NTR1), high affinity and most predominant, and neurotensin receptor 2 (NTR2), low affinity receptor—, and/or to an intracellular type I receptor—neurotensin receptor 3 (NTR3) [74].

NT displays pro-inflammatory properties by stimulating vasodilation, vascular permeability, immune cell migration, and phagocytosis [75–77]. In addition, NT was able to induce IL-8 expression via NF-ƙB and ERK pathways in human colonocytes [78] and has been implicated in the pathophysiology of acute colonic inflammation and intestinal angiogenesis [79–81]. However, NT also demonstrates protective effects in inflammatory conditions, as shown by its ability to modulate intestinal inflammation and stimulate healing following experimentally induced colitis [82, 83]. Together, these findings suggest an important immunomodulatory role for this neuropeptide. Moreover, NT was found to promote migration of microglial cells in an in vitro cerebral wound healing model [84]. Furthermore, the proliferative effects of NT have been shown in both normal and malignant cells.

Most NT studies have focused on the central nervous system or gastrointestinal tract and little is known about NT signaling in diabetes, particularly in diabetic skin and/or wounds. Interestingly, recent studies have suggested a role for NT in the development of diabetes. Fasting plasma levels of pro-neurotensin were associated with increased risk of diabetes incidence in human subjects [85], and were also elevated in both obese and insulin-resistant subjects [86]. In addition, increased levels of NT were observed in the pancreas of obese (ob/ob) mice and in the intestine of both ob/ob and diabetic (db/db) mice, and correlated with insulin deficiency [87, 88]. However, other studies reported no differences in NT levels between nondiabetic, diabetic lean and diabetic obese subjects, or between obese type 2 diabetic mice and their respective lean controls [89, 90].

Recent studies have demonstrated that while inducing inflammation under homeostatic conditions, NT downregulates the inflammatory responses of skin dendritic cells, macrophages, and fibroblasts when cells are previously exposed to pro-inflammatory and/or hyperglycemic conditions [91–93]. Such findings highlight the role of NT as an immune and inflammatory modulator. In addition, NT treatment of cells previously exposed to LPS yielded different results than pretreatment with NT followed by LPS treatment [91], suggesting that timely release of endogenous NT is crucial for proper wound healing responses. Of interest, hyperglycemic conditions reduced the expression of endogenous NT and its cell surface receptors NTR1 and NTR2 in mouse macrophages, and this was associated with a reduction in macrophage migratory capacity, whereas NT treatment was able to partially reverse the hyperglycemia-induced impaired cell migration [92]. In addition, hyperglycemia significantly reduced NT and NTR expression in a human keratinocyte cell line [94]. However, treatment of human keratinocytes cultured in high glucose conditions with NT did not significantly affect cell proliferation, migration, or cytokine release. Together these results suggest that NT does not have a direct effect on keratinocytes, but may work either via paracrine and/or autocrine effects on macrophages, dendritic cells, and fibroblasts.

More importantly, in vivo studies have shown that when topically applied to diabetic (and/or nondiabetic) mouse

wounds, chitosan-, collagen-, or alginate-based biomaterials delivering NT, either alone or in combination with SP, significantly accelerate healing [39, 95, 96]. Similarly to SP treatment, NT treatment induced the expression of pro-inflammatory cytokines TNF-α, IL-6, and KC (mouse homolog of human IL-8) in day 3 wounds of diabetic mice, while reducing it at day 10 post-wounding. In addition, NT treatment reduced MMP-9 gene and protein expression in the diabetic mouse wounds at the later stages of healing, and this was associated with an increase in fibroblast migration, as well as with an increase in the expression and deposition of collagen [95, 96].

In summary, NT improves diabetic wound healing via suppression of the prolonged and uncontrolled inflammatory response, and subsequent induction of the proliferative phase of healing. This seems to be achieved by regulating the timely expression of pro-inflammatory cytokines, stimulating fibroblast migration, and modulating extracellular matrix (ECM) remodeling, all of which are compromised in diabetic wounds and required for proper healing.

Calcitonin Gene-Related Peptide and Diabetic Wound Healing

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide widely distributed in the central and peripheral nervous systems, and also present in non-neuronal tissues. The receptors for CGRP and related peptides are calcitonin receptor-like receptors (CLR) linked to an essential receptor activity modifying protein (RAMP), which is required for full functionality. In the peripheral nervous system, CGRP is co-localized with SP and released from capsaicin-sensitive peripheral afferent neurons, and therefore is implicated in pain signaling. In addition, CGRP is one of the most potent peripheral microvascular vasodilators and has been shown to have cardioprotective effects [97–99].

Most studies involving diabetes and CGRP focus on the cardiovascular system. Diabetes was found to reduce the expression of CGRP and its receptors in rodents, and to reduce CGRP mediated vasodilation in rats [100–107]. In addition, CRGP circulating levels were reduced in human subjects with diabetes and cardiovascular disease [108]. In support of such findings, CGRP gene transfer was shown to have protective effects in a diabetic mouse model of ischemic-reperfusion injury [109]. Conversely, a study in human obese nondiabetic subjects detected a modest increase in CGRP levels compared to lean controls [110], and a similar increase was also observed in pre-obese Zucker rats [111]. Additionally, mice deficient in αCGRP are protected against diet-induced obesity, show improved glucose tolerance and increased insulin sensitivity [112], suggesting that CGRP inhibition may prove beneficial in the treatment of obesity and insulin resistance. In conclusion, the impact of diabetes on CGRP and the role of CGRP in diabetes are complex and require further investigation. Nonetheless, it is known that diabetic peripheral neuropathy leads to loss of CGRP-containing sensory nerves [113–115].

CGRP is released in the skin from sensory afferents, and can also be secreted by keratinocytes and immune cells, including monocytes/macrophages, and Langerhans cells [4]. There is evidence that CGRP participates in wound healing, as CGRP administration increased blood flow and flap survival in a rat skin-flap model [116], and accelerated healing in a blister model of the rat hind footpad [117], whereas deficiency in CGRP, deficiency in CGRP receptors, or pharmacological antagonism of CGRP impaired wound healing [118–121]. The beneficial effects of CGRP in wound healing are likely related to vasodilation, induction of VEGF release, and angiogenesis [117, 121–123]. In addition, CGRP promoted wound healing of human bronchial epithelial cells by stimulating cell survival, proliferation, and migration via activation of PKC and MAPK pathways [124] .

The role of CGRP in inflammation and immunity is also complex, as it may increase the flow of immune and inflammatory cells to the site of injury following vasodilation and stimulate pro-inflammatory cytokine release, or suppress pro-inflammatory mediator release via cAMP [119]. Namely, whereas CGRP can increase the release of pro-inflammatory cytokines including IL-1, IL-8, IL-6, and TNF-α [125–128], as well as stimulate macrophage phagocytic activity [129], it also has the ability to inhibit lymphocyte differentiation, proliferation, and IL-2 production [130–132], modulate Langerhans cell antigen-presenting function [133], suppress pro-inflammatory Th1, and induce regulatory Th2 responses [134]. Interestingly, a recent study suggested that CGRP induces a regulatory phenotype in TLR4-stimulated macrophages [135].

Together, these findings clearly indicate a role for CGRP as an immunomodulator and inflammation regulator. Although the properties of CGRP suggest a potential candidate for the treatment of diabetic wounds, the knowledge on this subject is very limited and new studies are needed to test this hypothesis.

Alpha-Melanocyte-Stimulating Hormone and Diabetic Wound Healing

Alpha-Melanocyte-stimulating hormone (α-MSH) is a tridecapeptide that belongs to the melanocortin family and derives from the melanocortin precursor pro-opiomelanocortin (POMC), which is mainly expressed in the pituitary gland but can be found in a variety of tissues [136]. Five melanocortin receptors (MC1-5R) have been identified to date, and shown to be involved in the regulation of many physiological phenomena including skin pigmentation (MC1R), cortisol production

(MC2R), food intake and energy metabolism (MC3R and MC4R), and temperature regulation (MC5R) [137–143]. Various skin cell types, including melanocytes, keratinocytes, fibroblasts, and endothelial cells produce α-MSH and express melanocortin receptors [144–146]. Like α-MSH, the enzyme that catalyzes its degradation—prolylcarboxypeptidase (PRCP)—is expressed in the central nervous system and in a variety of peripheral tissues, including the skin.

In diabetes, α-MSH appears to have protective effects. In particular, it reduces weight gain, adiposity, and hepatic fat accumulation while stimulating muscle glucose uptake and increasing energy expenditure in mouse models of obesity [147, 148], as well as protects retinal vascular endothelial cells against oxidative stress and apoptosis in a rat model of diabetes [149]. Of interest, studies have shown reduced expression of POMC in the hypothalamus and pituitary of streptozotocin-induced diabetic rats [150, 151], increased plasma levels of the enzyme PRCP in obese and/or diabetic human subjects [152], and even suggested a role for α-MSH deficiency in the development of type 2 diabetes [153].

α-MSH is also known for its protective effects in inflammatory conditions, including colitis, brain and pulmonary inflammation, transplantation, as well as skin inflammatory diseases such as urticaria and psoriasis [154–164]. Its anti-inflammatory properties are vast and target multiple cells such as lymphocytes, monocytes and macrophages, mast cells, endothelial cells, fibroblasts, and keratinocytes, where α-MSH inhibits the NF-ƙB pathway. Namely, α-MSH suppresses proliferation of stimulated lymphocytes and modulates its activity, inducing a regulatory phenotype [165, 166]. In addition, α-MSH inhibits monocyte adhesion to the vascular endothelium and reduces TNF-α release from LPS-stimulated human monocytes in culture, while increasing IL-10 in both human peripheral blood monocytes and cultured monocytes [167–169]. Of note, such effects were achieved with low doses (range of 10^{-10} to 10^{-17} M). α-MSH also attenuates the expression of IFN-γ and nitric oxide in LPS-stimulated murine macrophages [170, 171] and reduces histamine, IL-1β and TNF-α release from IgE-stimulated bone marrow-derived mouse mast cells [172]. In human dermal fibroblasts and endothelial cells, it regulates the expression of IL-8 [173, 174] and in human keratinocytes increases expression of anti-inflammatory IL-10 [175].

A study has suggested that α-MSH inhibits angiogenesis [176], which is crucial for proper wound healing. However, α-MSH restored H_2O_2-induced inhibition of wound restitution in a rat intestinal epithelial cell scrape wound model, suggesting a potential role in healing involving epithelial cells [177]. In fact, topical application of the C-terminal tripeptide (KPV) sequence of α-MSH to a rabbit corneal wound model confirmed positive healing outcomes [178]. More importantly, a recent study showed that pretreatment with intraperitoneal α-MSH ameliorates cutaneous wound healing in adult mice [179]. This was accompanied by a reduction in the number of leucocytes and mast cells at days 3 and 7 post-wounding, and by a reduction of scar area as well as improvement of dermal architecture at days 40 and 60 post-wounding [179]. Despite its apparent anti-angiogenic effect, the beneficial effects of α-MSH in acute noncomplicated wound healing, as well as its protective effects against inflammation and diabetes, make it a promising candidate for the treatment of diabetic wounds.

The abovementioned studies indicate that neuropeptides play an important role in wound healing, mostly by promoting angiogenesis and modulating the immune and inflammatory responses to injury. Since diabetes reduces the cutaneous expression of neuropeptides, impairs wound neovascularization, and causes wounds to become stalled in the inflammatory phase, these neuropeptides have great potential to ameliorate the healing of diabetic wounds. While the beneficial effects of some of these neuropeptides, such as SP and NT, have been confirmed in experimental models of diabetic wound healing, others still require investigation (Table 8.1).

Inflammation and Diabetic Wound Healing

Inflammation is essential in the wound healing process, but in order to achieve proper healing, the inflammatory response must be tightly regulated in time, space, and magnitude. In

Table 8.1 Summary of neuropeptide effects on angiogenesis and inflammation, as well as on nondiabetic and diabetic cutaneous wound healing

Neuropeptide effects	Angiogenesis	Inflammation	Nondiabetic skin wound healing	Diabetic skin wound healing
Substance P (SP)	Promotes angiogenesis [16–18]	Modulates inflammation [13–15, 29, 34–36]	Improves healing [19, 29]	Improves healing [23, 29, 34]
Neuropeptide Y (NPY)	Promotes angiogenesis [56, 57]	Modulates inflammation [58–64]	Needs investigation	Needs investigation
Neurotensin (NT)	Promotes angiogenesis [80, 81]	Modulates inflammation [75–79, 82, 83, 91–93]	Improves healing [95]	Improves healing [95, 96]
Calcitonin gene-related peptide (CGRP)	Promotes angiogenesis [117, 121–123]	Modulates inflammation [119, 125–135]	Improves healing [116, 117, 121]	Needs investigation
Alpha-melanocyte-stimulating hormone (α-MSH)	Inhibits angiogenesis [176]	Suppresses inflammation [154–175]	Improves healing [179]	Needs investigation

physiological conditions, skin injury causes a rapid onset of acute and self-resolving inflammation, with timely recruitment of immune cells from circulation—neutrophils and monocyte-derived macrophages—, as well as controlled activation of tissue-resident immune cells, such as mast cells (MCs), T cells, and Langerhans cells [180–184]. Besides controlling and fighting infection, acting as phagocytic agents, and/or as antigen-presenting cells, immune cells also release a cocktail of cytokines, chemokines, and growth factors, essential for fibroblast and endothelial cell proliferation and migration, ECM production, granulation tissue formation, and angiogenesis [181, 185–190]. Therefore, this acute inflammatory cell influx is crucial for the progression to the proliferative phase of healing, and pathological conditions that interfere with this self-limited process can result in a chronic nonhealing wound.

Systemic and local chronic inflammation, altered ECM deposition, and impaired wound neovascularization, as a result of an imbalanced secretion of cytokines, matrix metalloproteinases (MMPs), and growth factors, have been implicated in the pathophysiology of DFUs [30, 191]. Diabetes is characterized by sustained hyperglycemia and chronic elevation of pro-inflammatory mediators, leading to a chronic low-grade inflammation, with impaired cellular defense mechanisms that fail to mount an acute response to injury. This delays the formation of mature granulation tissue and reduces the wound tensile strength [192]. Due to the chronic pro-inflammatory environment, there is an imbalance between wound MMPs and their inhibitors, which also contributes to poor formation of new connective tissue [32, 193–195]. In addition, analysis of the fluid of diabetic wounds from both animal models and human subjects has shown insulin-degrading activity, which in turn has been correlated with the levels of hemoglobin A1c (HbA1c) [196], suggesting a straight relationship between sustained hyperglycemia and the wound proteolytic environment. Moreover, macrophage efferocytosis is impaired in diabetic wounds, resulting in increased apoptotic burden and imbalanced inflammatory status with higher levels of pro-inflammatory cytokines and lower levels of anti-inflammatory mediators [197, 198]. Neutrophils also show reduced chemotactic and phagocytic activities, rendering the wounds more prone to infection [199, 200]. In fact, diabetic patients have over 50% higher risk of wound infection and are also more likely to develop biofilms compared to nondiabetic subjects [201–204]. Naturally, infection and biofilm formation further hinder the healing process.

In summary, contrasting with normal wound healing, where inflammation occurs in a sequential, regulated, and self-resolving manner, in diabetic wounds the immune and inflammatory responses are prolonged and noneffective. As a consequence, diabetic wounds become stalled in a chronic inflammatory state and fail to progress to the proliferative and reparative phases of healing. Further understanding of this process could help identify and develop new therapeutic strategies.

As emphasized earlier in this chapter, whereas neuropeptides play an important role in wound healing, namely by regulating the inflammatory response to injury, their skin expression is reduced in diabetes, and therefore exogenous application of neuropeptides may be a beneficial strategy in the treatment of diabetic wounds. However, due to the highly proteolytic environment of the diabetic wound, neuropeptide delivery should ensure protection against rapid inactivation. For example, biomaterials such as alginate-, chitosan-, and collagen-based materials serve as vehicles for sustained delivery of neuropeptides to the wounds and prevent rapid neuropeptide degradation at the wound site [39, 95, 96]. An alternative strategy in the treatment of diabetic wounds is to directly target immune cells to (1) prevent increased baseline skin inflammation, (2) promote the acute inflammatory response, and/or (3) contribute to proper resolution of the inflammatory phase and progression to the proliferative phase.

Mast Cells and Diabetic Wound Healing

Mast cells (MCs) are immune cells that originate from hematopoietic pluripotent stem cells in the bone marrow [205–208]. Committed MC-progenitors are released into the bloodstream and subsequently home to virtually every organ in the body, where they differentiate and mature under the influence of tissue-specific growth factors and cytokines, giving rise to distinct phenotypes in different tissues [206, 207, 209]. Mature MCs are more abundant in tissues interfacing with the external environment, including the skin, the respiratory and gastrointestinal tracts, as well as in proximity with blood vessels [210, 211].

A particular feature of mature MCs, especially those residing in the skin and other connective tissues, is that their cytoplasm is filled with numerous granules, where preformed mediators are stored [212]. MC activation results in rapid degranulation (5–30 min) [213] with exocytosis of various preformed mediators, including biogenic amines—mostly histamine and serotonin; enzymes—beta-hexosaminidase, tryptase, and chymase; proteoglycans—serglycin proteoglycan (SGPG), heparin, chondroitin sulfate, and hyaluronic acid; as well as the preformed cytokine TNF-α [214, 215]. MC activation also induces de novo synthesis and delayed release (12–24 h later) of various cytokines and chemokines [214, 216], including interferon-α (IFN-α), TNF-α, several interleukins (IL-1β, IL-3, IL-4, IL-5, IL-6, IL-8, IL-13), and chemokine MCP-1, as well as growth factors such as stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF), nerve growth factor (NGF),

and VEGF [214, 217]. Of note, some of these biological mediators can be secreted to the extracellular environment through selective or differential release, a process that occurs independently from degranulation [218].

MCs are mostly known as the effector cells in immunoglobulin E (IgE)-mediated allergic responses [219–222]. However, they also participate in several other physiological and pathophysiological processes, and there is growing interest in the role of MCs in nonallergic immune and inflammatory responses, including cutaneous wound healing [211, 223–231]. In the skin, MCs are abundant and strategically located in the vicinity of blood vessels and sensory nerves [232]. In fact, there is a bidirectional communication between MCs and primary sensory neurons, as neuropeptides such as substance P (SP) [233] and neurotensin (NT) [234] stimulate/activate MCs, and MC mediators in turn regulate neuropeptide release [235]. Unlike the IgE-mediated process, neuropeptide-induced MC activation occurs through different G-protein coupled receptors [236, 237], and also via receptor-independent mechanisms by direct or indirect activation of G proteins [238–240]. Interestingly, neuropeptides such as corticotropin-releasing hormone (CRH) and SP can cause selective release of VEGF from MCs without degranulation [241, 242].

Skin MC activation occurs soon after tissue injury, but the exact mechanisms are not fully understood [243, 244]. Pathogens, lipopolysaccharides (LPS), other pathogen products, and cytokines [211, 245], as well as pain signals, such as SP [246], and mechanical stress [247] may be involved. MCs have been implicated in all phases of wound healing [217, 231, 244, 247–250]. More specifically, MCs induce vascular permeability and participate in the fibrin clot formation, but also prevent excessive clotting, as they secrete tryptase-heparin complexes that degrade the excess fibrinogen [251, 252]. In addition, MCs contribute to inflammation by recruiting neutrophils to the wound site [183, 253–255], as well as releasing chemokines, cytokines, histamine, and other mediators that activate tissue-resident macrophages [256]. Moreover, MCs promote the proliferative phase of wound healing as they stimulate proliferation and migration of several skin cell types, namely fibroblasts, endothelial cells, and keratinocytes [253, 257–259], induce vascular growth and angiogenesis [260–263], and participate in ECM remodeling [248]. Finally, MCs participate in the maturation phase of healing by stimulating wound contraction [264–270] and scarring [271–273].

While most in vivo studies have reported an important role for MCs in normal wound healing [247, 248, 253, 255, 260], other studies failed to confirm it [274–276]. Although the reasons for such discrepancy are not clear, the use of different mouse models or different wound healing models may play a role. Given the heterogeneity of mature MCs, it is possible that some models of MC deficiency do not lack the full spectrum of MCs, whereas others may have additional underlying defects. On the other hand, the use of splinted wounds [275] may explain the contradictory results observed. Another study that employed splinting has shown robust abnormalities in important parameters of wound healing, including cell proliferation, angiogenesis, tissue granulation, and collagen maturation, despite a lack of difference in wound closure [247]. Moreover, other authors have raised concerns that splinting may alter the wound healing phenotype due to mechanical changes [277].

Despite the large number of reports on mast cells and normal wound healing, few studies have investigated the role of MCs in diabetic wound healing. Interestingly, however, MCs have been implicated in insulin-induced lipoatrophy [278], obesity, and type 2 diabetes [279, 280], namely through IL-6 and IFN-γ release. Additionally, elevated plasma levels of MC proteases and MC degranulation activator IgE have been suggested as inflammatory markers and risk factors of human prediabetes and diabetes [281]. Others studies have suggested a role for MCs in the pathophysiology of type 1 diabetes. More specifically, MCs have been implicated in both the development of type 1 diabetes in the spontaneously diabetic BioBreeding rat [282], and the immune-mediated beta cell alterations that occur in human type 1 diabetes [283]. However, MCs can also have protective effects against streptozotocin-induced diabetes in mice, namely by increasing the pool of regulatory T cells and decreasing IL-17 producing T cells in pancreatic lymph nodes [284], whereas MC deficiency has been shown to worsen type 1 diabetes and its complications [284, 285].

A recent study used streptozotocin-induced diabetic mice and nondiabetic mice to create excisional wounds and evaluate the healing process with focus on MC numbers [286]. Despite not having found significant differences in wound closure between the diabetic and nondiabetic groups, perhaps due to the short duration of diabetes (4 weeks), neovascularization in the proliferative phase and vascular regression in the remodeling phase were impaired in the diabetic mice. Of interest, MC accumulation in the diabetic wounds was delayed when compared to their nondiabetic counterparts [286].

We have recently reported increased MC degranulation in unwounded forearm and foot skin from diabetic human subjects, which was associated with increased local and systemic inflammation (Fig. 8.4) [287]. Similarly, increased MC degranulation was also observed in dorsal skin from streptozotocin-induced diabetic mice, when compared to their nondiabetic controls. In addition, we have shown that whereas nondiabetic mouse skin MCs undergo considerable degranulation after injury, an observation previously reported by others [243, 244, 247, 255], in diabetic mice MC degranulation does not further increase post-wounding (Fig. 8.5). This failure to induce MC degranulation post-wounding is consistent

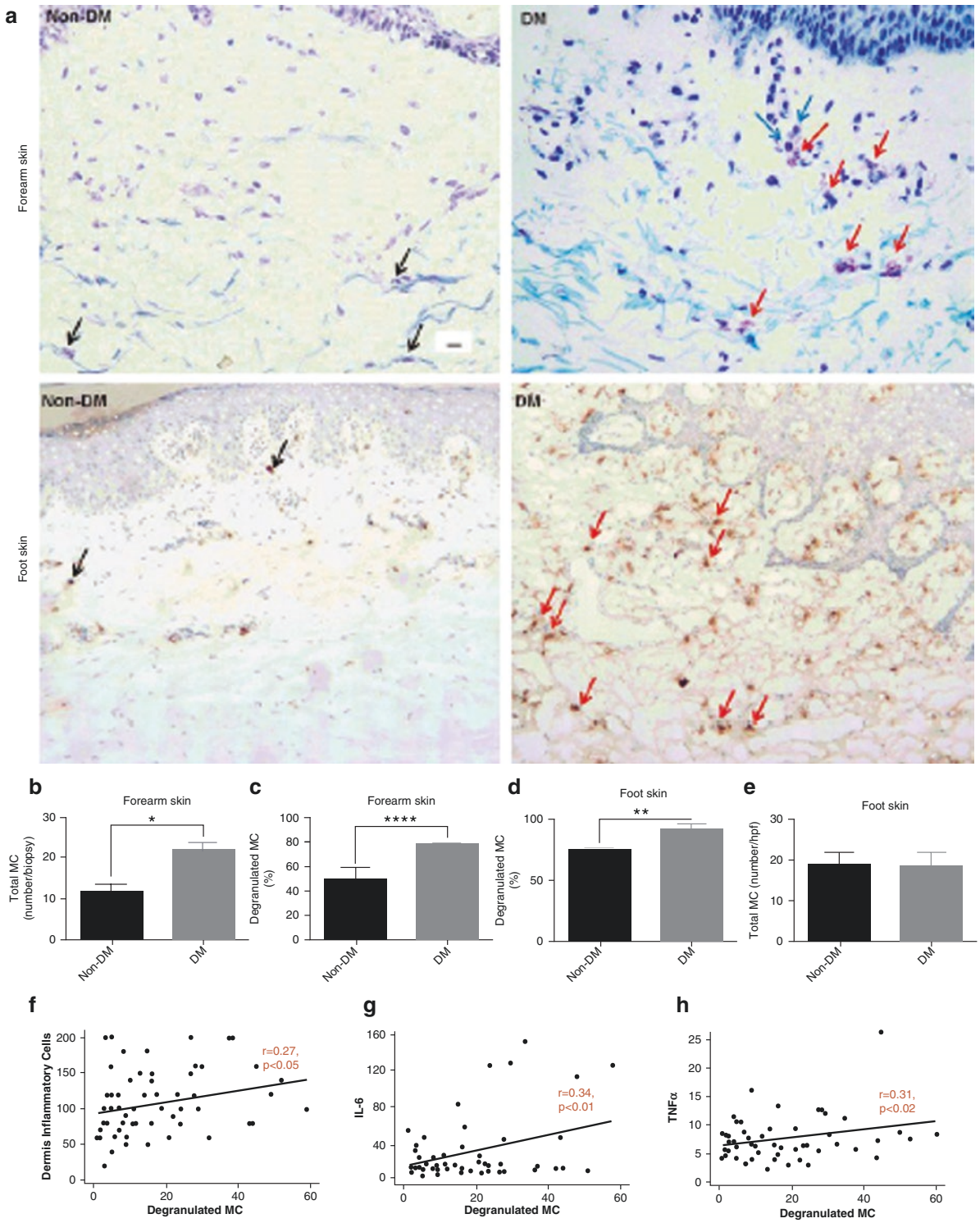

Fig. 8.4 Skin mast cell (MC) degranulation is increased in patients with diabetes and is associated with inflammation. (**a**) Representative images of toluidine blue-stained MCs in forearm skin (top panel) and of tryptase-immunostained MCs in foot skin specimens (bottom panel) from subjects without (Non-DM) and with diabetes mellitus (DM). Scale bar: 10 μm. Black arrows show nondegranulated MCs, and red arrows show degranulated MCs. Degranulated MCs were in proximity with inflammatory cells (blue arrows). The (**b**) total number and (**c**) percentage of degranulated MCs stained with toluidine blue were increased in forearm skin specimens from patients with diabetes. (**d**) MC degranulation was also increased in foot skin specimens from subjects with diabetes stained with tryptase, while (**e**) the total number of MCs was not different. $*p < 0.05$, $**p < 0.01$, and $****p < 0.0001$. A positive correlation was observed between degranulated MCs and (**f**) the dermis inflammatory cells, and the serum levels of (**g**) IL-6 and (**h**) TNF-α. Copyright© American Diabetes Association. Reprinted from Tellechea et al., Mast Cells Regulate Wound Healing in Diabetes, Diabetes 2016 Jul; 65 (7):2006–2019, with permission from The American Diabetes Association

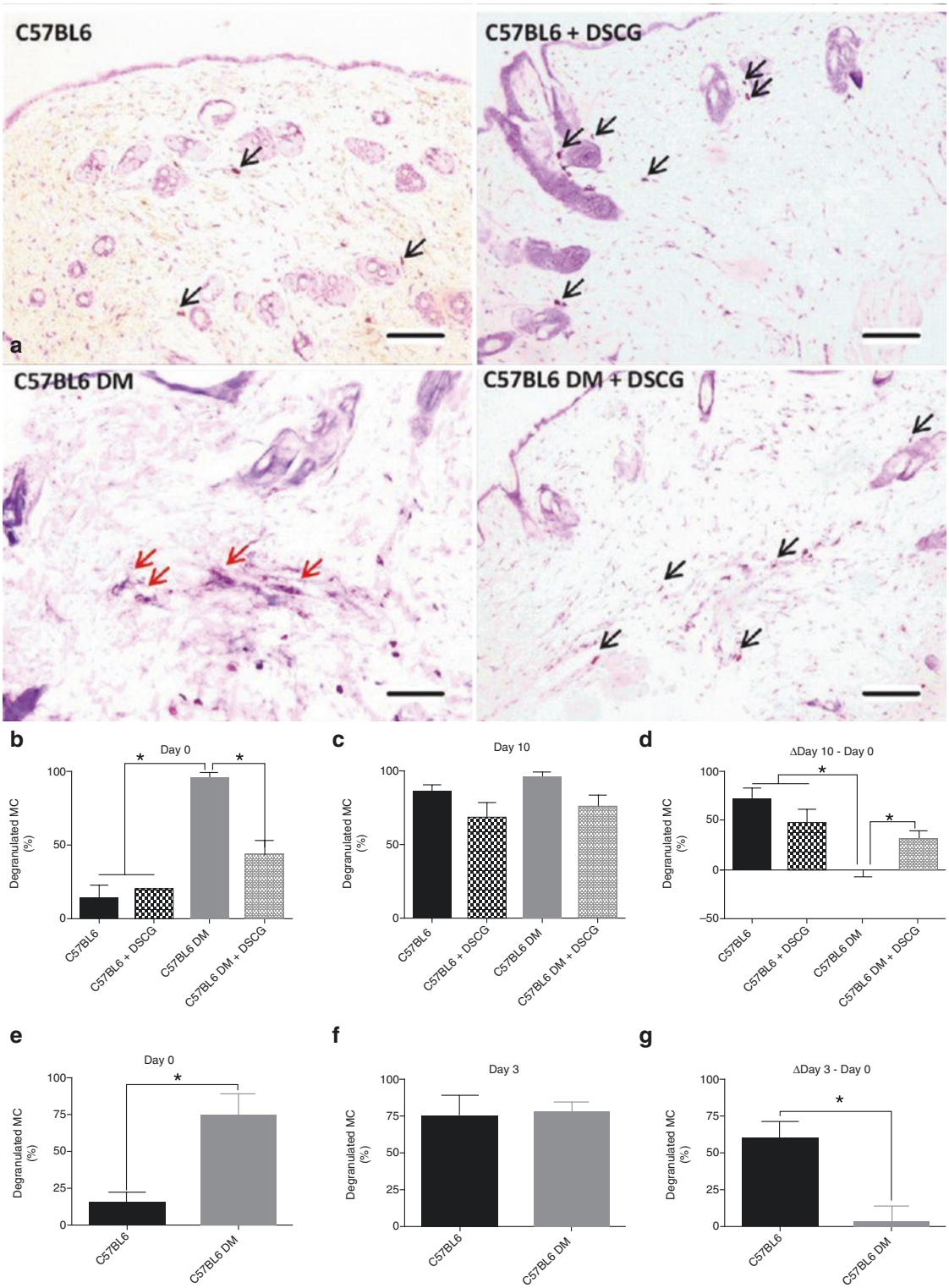

Fig. 8.5 Mast cell (MC) degranulation is increased in unwounded skin of diabetic (DM) mice and fails to further increase after wounding. (**a**) Representative images of nondegranulated (black arrows) and degranulated (red arrows) MCs in day 0 skin biopsy specimens from WT C57BL/6J nondiabetic and diabetic (DM) mice, untreated and pretreated with the MC degranulation inhibitor DSCG. Scale bar: 100 μm. (**b**) MC degranulation was increased in diabetic mice at day 0 and DSCG pretreatment reduced it. (**c**) Nondiabetic mice showed increased MC degranulation at day 10 when compared with day 0, but there were no changes in DM mice between days 0 and 10. As a result, there were no differences among the various groups at day 10. (**d**) When the difference between days 10 and 0 was calculated, a significant increase was noticed in nondiabetic mice, irrespective of treatment. No difference was observed in the DM mice, but this difference was restored in the DSCG-treated diabetic mice. (**e–g**) Similar results were observed in a different set of nondiabetic and diabetic mice that were studied at day 3 post-wounding. Thus, (**e**) MC degranulation was also increased in the skin in diabetic mice on day 0, (**f**) but was not different at day 3, resulting in (**g**) failure to increase MC degranulation from day 0 to day 3 post-wounding in diabetic mice. *$p < 0.05$. Copyright© American Diabetes Association. Reprinted from Tellechea et al., Mast Cells Regulate Wound Healing in Diabetes, Diabetes 2016 Jul; 65(7):2006–2019, with permission from The American Diabetes Association

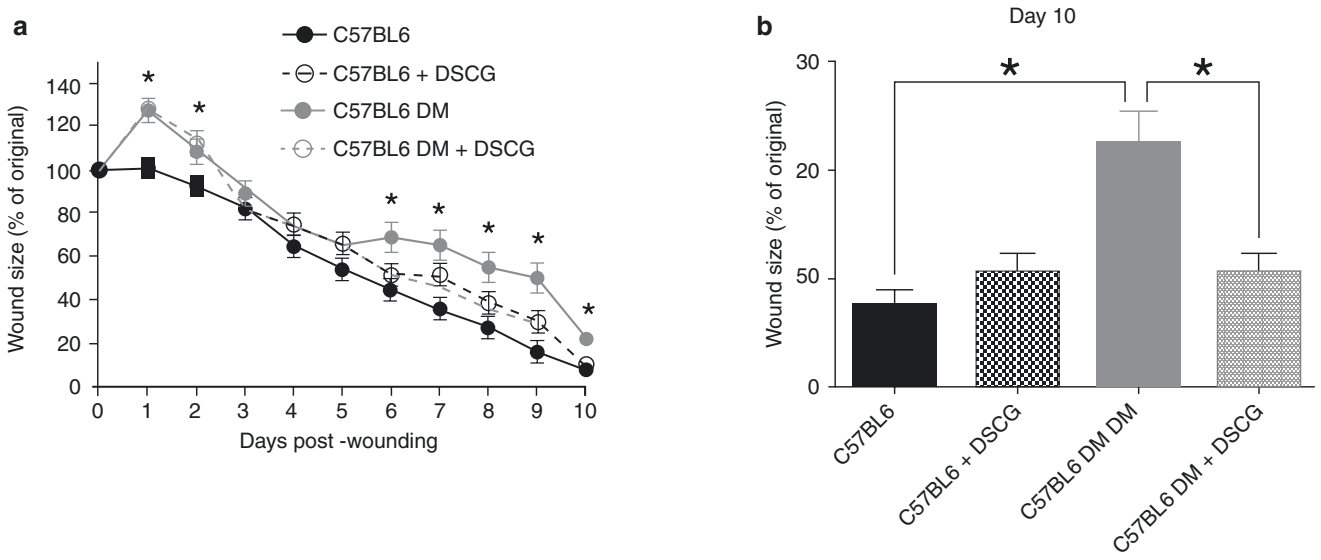

Fig. 8.6 Blocking pre-wounding mast cell (MC) degranulation with DSCG accelerates wound closure in diabetic (DM) mice. (**a**) Wound healing progress over a 10-day period in wild-type (WT) C57BL6 nondiabetic and diabetic (DM) mice, non-treated and DSCG pretreated. Wound healing was delayed in DM mice compared to nondiabetic mice and DSCG pretreatment accelerated it from days 6 to 10 post-wounding. DSCG had no effect on nondiabetic mouse wound healing. (**b**) At day 10 post-wounding, pretreatment with DSCG in diabetic mice achieved similar wound closure to nondiabetic mice . Data represent mean ± SEM. *$p < 0.05$. Copyright© American Diabetes Association. Adapted from Tellechea et al., Mast Cells Regulate Wound Healing in Diabetes, Diabetes 2016 Jul; 65(7):2006–2019, with permission from The American Diabetes Association

with the inability to mount an acute inflammatory response to injury observed in diabetic animals [25, 29], and may contribute to poor healing.

More importantly, treatment with the MC degranulation inhibitor disodium cromoglycate (DSCG) prior to wounding significantly accelerated wound closure in diabetic mice, achieving an outcome comparable to that of nondiabetic mice (Fig. 8.6). The observed improvement in wound healing was associated with M2 macrophage polarization, with stimulation of wound neovascularization, as well as with an elevation in VEGF (known to be reduced in diabetic wounds), and a reduction in MMP-9 (elevated in chronic wounds) local expression at day 10 post-wounding [287]. Such findings suggest that blocking pre-wounding MC degranulation ameliorates diabetic wound healing by suppressing the chronic inflammation observed in diabetes, and promoting angiogenesis.

Of interest, whereas topical treatment with SP accelerated wound healing in both nondiabetic and diabetic mice, confirming previous findings [29], it did not affect wound closure in MC-deficient mice [287], suggesting that the beneficial effects of SP in wound healing are at least partly mediated by MCs.

In summary, the results emerging from this study suggest that strategies to prevent/inhibit chronic MC degranulation may ameliorate wound healing in diabetes. In light of the recent controversy regarding the role of MCs in normal, noncomplicated wound repair, further studies evaluating MC function in diabetic wound healing are of major importance to test the validity of the abovementioned findings, and to further examine the underlying mechanisms.

In Vivo Models of Diabetic Wound Healing: Focus on Neuropeptides and Mast Cells

The previous sections of this chapter highlighted the role of neuropeptides and mast cells in wound healing. Both in vitro systems and animal models are useful tools to evaluate their potential as therapeutic targets for diabetic wound healing, as they help us understand their mechanisms of actions and test their efficacy.

Wound repair is a highly complex and dynamic process, encompassing a series of coordinated and overlapping phenomena, which involve multiple cell types with autocrine and paracrine effects, and are affected by the extracellular environment. Therefore, it cannot be recapitulated by simple single cell assays. In an effort to overcome some of the limitations of such assays, in vitro organotypic models have been fabricated, including a 3D full-thickness skin equivalent that is composed of not only epidermis and dermis, but also a layer of hypodermis containing blood vessels, nerves, and fibroblasts, which provide support to the epidermis and dermis [288]. Whereas such models enable exploration of the cross talk between different cell types, —mostly fibroblasts and keratinocytes—, they still lack the complexity of in vivo models, namely, the immune and inflammatory components of wound healing. Ex vivo skin explants have also been developed and optimized to study wound healing processes [289–291]. But once again they fail to fully recreate the wound environment present in their in vivo counterparts, and cannot identify potential off-target or systemic effects of the therapeutics tested. Therefore, and while research continues to make an effort to use alternative experimental models,

animal models are currently required to better understand the healing processes.

Rodents are the most widely used in vivo models of diabetic wound healing, due to their economic feasibility, easy manipulation, and relatively short reproduction times. Another advantage of rodents, particularly mice, is the availability of genetically modified models, which allow studying the role of a particular cell type or molecule. Mice and rats can be rendered diabetic via Streptozotocin (STZ), or, less commonly, Alloxan Monohydrate, drugs that destroy the beta cells in the pancreas and cause type 1 diabetes. The leptin receptor-deficient (db/db) mouse is another commonly used model for diabetic wound healing [292, 293]. Db/db mice spontaneously develop obesity and subsequently type 2 diabetes at 4–6 weeks of age, with hyperinsulinemia and hyperlipidemia.

It is important that chronic diabetes and its complications, namely diabetic peripheral neuropathy—the most common complication of diabetes affecting approximately 50% of the patients [294–296] and a major risk factor for DFU in humans [1, 297]—, have been established prior to creating cutaneous wounds, especially when studying neuropeptides. The minimum duration of diabetes depends on the animal model and the features of neuropathy to be studied. In case of the STZ-induced diabetic rat, nerve conduction velocity slowing and impaired sensory responses occur as early as 2–4 weeks post-STZ treatment; in the STZ-diabetic mice such changes occur within 2–8 weeks of diabetes, whereas in the db/db mice they start at 4–8 weeks of diabetes, and progress over time [298]. Nonobese diabetic (NOD) mice and Akita mice, two genetically modified mouse models of type 1 diabetes, have also been used in wound healing studies [299–302]; however, there is inconsistent information about the neuropathy status in these models [298].

Despite being the most studied models of diabetic wound healing, both the STZ-induced diabetic and the db/db murine models have limitations. While some authors argue that type 1 diabetes models are not ideal to study DFU, others claim that the wound healing impairment observed in db/db mice may be more related to other underlying abnormalities, such as obesity, different skin properties, and leptin pathway disruption, than to diabetes. Other polygenic type 2 diabetic strains, namely the NONcNZO10 [303] and the TALLYHO [304] mouse models, have been developed and reported to have wound healing defects.

Genetically modified rat models of diabetic wound healing are also available. They include the models of type 2 diabetes, such as the Goto-Kakizaki (GK) nonobese and the JCR:LA-cp/cp obese rats [305–307]. Although Otsuka Long-Evans Tokushima Fatty (OLETF) rats are typically models of corneal diabetic wound healing, they have also been used to study cutaneous diabetic wounds [308].

Of interest, and as mentioned in the previous sections of this chapter, multiple mouse models of neuropeptide-, neuropeptide receptor-, and mast cell-deficiency have been developed and used in wound healing studies. Examples of murine models that lack specific neuropeptides or neuropeptide receptors involved in wound healing are the tachykinin 1 *knock-out* (TAC1KO) mice that lack substance P (SP) or neurokinin A [29], neurokinin 1 receptor *knock-out* (NK1RKO) mice that lack the high affinity SP receptor [29], and neuropeptide Y (NPY) 2 receptor *knock-out* mice [71]. Other neuropeptide-deficient models exist, but have yet to be used in wound healing studies.

Multiple models of mast cell (MC)-deficiency are available and have been employed to study the role of MCs in wound healing. The most studied MC-deficient models include Kit^{W/W^v} and $Kit^{W\text{-}sh/W\text{-}sh}$ mice [247, 253, 260, 275, 287]. The Kit^{W/W^v} mouse model has truncated W and point-mutated Wv alleles, resulting in reduced Kit expression, severe MC deficiency, and other non-MC-related abnormalities, including neutropenia, anemia, and lack of certain subpopulations of germ cells and melanocytes. The $Kit^{W\text{-}sh/W\text{-}sh}$ model possesses an inversion mutation upstream of the c-kit promoter region, leading to a selective reduction in Kit expression. Therefore, in contrast with the Kit^{W/W^v} strain, the $Kit^{W\text{-}sh/W\text{-}sh}$ mouse has normal levels of other differentiated hematopoietic and lymphoid cells [309, 310]. However, these mice also present other problems, likely related to the reduced kit expression, such as splenic myeloid and megakaryocytic hyperplasia. In order to overcome such limitations, researchers have either performed MC reconstitution experiments [253], or developed and used new strains of Kit-independent MC-deficient mice, including Cre recombinase-mediated carboxypeptidase A3 (Cpa3Cre) [274] or mast cell protease 5 (Mcpt5Cre) eradication [276]. However, the observed wound repair phenotypes differed between the models used.

Nonetheless, despite the inconsistent results in normal noncomplicated wound healing, our recent findings implicate MCs in diabetic wound healing, since skin MC degranulation is increased in both diabetic human subjects and STZ-induced diabetic mice [287]. Of note, the same study also suggests that pharmacological blockade of chronic MC degranulation, rather than depletion of MCs, may be a useful tool to further evaluate diabetic wound healing, and to potentially develop new treatments.

Not only different mouse models, but also different wound models have resulted in different wound healing outcomes, complicating the predictability for translation into humans. The most common wound models are full-thickness excisions or full-thickness incisions, created on the shaved dorsal skin of the animal. Wounds can then be left exposed (healing by secondary intention, which comprises greater wound contraction), dressed (dressings have the particularity of preventing excessive "dry" conditions and creating a "moist" environment [311]), splinted (with the goal of minimizing contraction [312]), or sutured (usually following incisional wounds). While different models are used to address different

scientific questions, for example, incisional sutured wounds are often used as a tool for investigating scarring, there is still little consensus regarding the ideal model to study diabetic wound healing.

A major caveat of the rodent models is their intrinsic anatomical and physiological differences to human skin and wound repair. Compared to humans, rodents exhibit higher epidermal appendage density with different hair follicles and a distinct hair growth cycle, have thinner epidermis and dermis, are "loose-skinned" animals, and heal more rapidly, mostly by contraction with less reepithelialization [313–315]. While splinting was developed as a strategy to minimize wound contraction in rodents [312], with the goal of making healing more comparable to humans, concerns that splinting may alter the wound healing phenotype due to the external mechanical tension have been raised [277]. Another drawback of mice and rats is the limited number of wounds that can be produced in each animal, often requiring the use of a larger number of animals per experimental condition.

The most commonly used large animals in wound healing studies are the rabbit and porcine models, and both heal more similarly to humans than rodents. Rabbits are usually made diabetic by Alloxan injections, and pigs by STZ. Multiple wounds can then be created either in the rabbit ear or in the porcine dorsum. Unlike rodents and similarly to humans, the rabbit ear skin is highly vascularized, and is involved in thermoregulation [316–319]. Contrary to mice and rats, the rabbit ear skin lacks panniculus carnosus, and therefore wound repair occurs mostly via reepithelialization rather than contraction. In addition, when creating wounds the cartilage is usually kept intact, contributing to stent the wound open and further minimizing contraction [24]. Another advantage of using the rabbit ear model to study DFUs is that it is relatively simple to create ischemic, neuropathic, and neuroischemic wounds, since the major blood vessels in the rabbit ear and nerves are easy to view, access, and manipulate. Ischemia is achieved by ligating the central and the rostral artery leaving the caudal artery and all the veins intact, whereas neuroischemia is created by ligating the central and rostral arteries along with central and rostral nerve resection [25]. This allows studying diabetic wounds that present the two most common long-term complications of diabetes, which are also major risk factors for DFU development—neuropathy and ischemia.

The porcine dorsal skin also resembles human skin in turnover time, epidermal and dermal layer thickness, skin appendages (with the exception of eccrine glands), well defined rete pegs and dermal papillae, dense elastic fibers, similar collagen structure, abundant subcutaneous adipose tissue, and lack of panniculus carnosus [314, 320, 321]. Differences include reduced dermal vascularity, absence of eccrine sweat glands, and distinct distribution of apocrine glands in the pig [320]. Transgenic pig lines have been recently generated, including a pig model of permanent neonatal diabetes by introduction of a mutation in the insulin gene [322]. The INS^{C94Y} diabetic pig manifested hyperglycemia soon after birth and significantly reduced β-cell mass at 4.5 months of age; however, no nervous tissue changes were observed during the first year [322], suggesting that the STZ-induced diabetic pig model is preferred to study diabetic wound healing.

It is clear that a high degree of phenotypic skin and wound repair exists between animal species, and that an ideal model for diabetic wound healing has yet to be developed. Despite their limitations, mice are likely to remain an essential tool to investigate the mechanisms of diabetic wound healing [323, 324]. However, the consensus is that findings should be reproduced in multiple animal models. Additionally, validation in human subjects significantly increases the translational potential, but also carries ethical concerns.

As the diabetes epidemic continues to rise, it is expected that its complications, namely chronic nonhealing DFUs will also increase in number, severity, and economic burden. Neuropathy and inflammation are gaining increasing attention, and studies on the role of neuropeptides and immune cells, such as mast cells and macrophages, in diabetes, obesity, cutaneous inflammation, and wound repair are emerging. Genetically modified mouse models and in vitro studies help researchers mechanistically probe the diabetic wound healing process, and confirmation in different animal models, such as the rabbit ear model, and in human skin specimens is extremely important to evaluate the relevance of the findings in pre-clinical studies. Neuropeptides and mast cell degranulation inhibitors are promising targets in the development of novel therapeutic strategies for diabetic wound healing, but further clinically driven translational research is required to validate them.

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Dermal Regeneration and Induction of Wound Closure in Diabetic Wounds

9

Giorgio Giatsidis, Dennis P. Orgill, and Ioannis V. Yannas

Abstract

This chapter reviews the biological and mechanical role of the extracellular matrix (ECM) in cutaneous wound healing. The multiplicity of viewpoints expressed in the literature, the lack of standards in evaluating research and surgical outcomes, and poor data quality have made our analysis challenging. We attempt to deliver a clear, objective analysis given these constraints. We highlight how chronic wounds impair the architecture of the ECM leading to a loss of structural and biochemical cues and halt healing. We also discuss how ECM scaffolds can be used therapeutically to repair or temporarily replace lost ECM, triggering healing, tissue regeneration, and ultimately effective wound closure. We further analyze biological characteristics, design principles, scientific evidence, and future challenges in the use of ECM scaffolds to treat chronic (diabetic) wounds. In particular, we debate the difference between ***bioactive ECM scaffolds*** that possess a regenerative capacity as stand-alone product and do not require pre-application (or simultaneous applications) of cells, and biological matrices designed as delivery methods of cells and growth factors. We discuss the elements of an "induced regeneration" theory that is organ nonspecific. Finally, we review the process behind the conception and development of a specific example of successful ECM scaffolds currently used for the treatment of diabetic chronic wounds.

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Role of the Extracellular Matrix (ECM) in Wound Healing

Wound healing is a complex process involving a highly orchestrated cascade of events: hemostasis, inflammation, proliferation, angiogenesis, and remodeling [1–5]. Dermal ECM plays a pivotal role in the regulation and modulation of each of these phases throughout healing [6–10]. Historically, the ECM has been considered a passive structure for cells without active functions. However, decades of clinical and preclinical research have highlighted the role of ECM as a key regulator and contributor of tissue activity during morphogenesis, physiological remodeling, and response to injury [6–14]. The ECM is a natural template for tissue remodeling: it gives structure and support to cells, provides biochemical and biomechanical cues, induces cell proliferation and differentiation, and facilitates cell communication [6–14]. During wound healing, the ECM acts as a dynamic microenvironment regulating a plethora of cellular processes [6–14].

Dermal ECM is the result of an accurate combination of proteoglycans (such as heparin sulfate) [15–17], proteins (such as collagens) [18–22], glycoproteins (such as fibronectin and laminin) [23, 24], and polysaccharides (such as hyaluronic acid) [25, 26]. These components define ECM's biological characteristics and determine its response to physiologic and pathologic stimuli [6–26]. ECM's components and structure regulate adhesion and migration of inflammatory cells (macrophages and neutrophils) or growth of vascular tissue (angiogenesis) [6–26]. For example, laminin fine-tunes cell adhesion, migration, proliferation, differentiation, and angiogenesis[23]; heparin sulfate induces proliferation of fibroblasts and endothelial cells, and functions as endogenous receptor for numerous growth factors and chemokines that further regulate cell differentiation or leukocytic migration and degranulation[17]; fibrillar collagens control fibroblast migration, proliferation, and activation [18–22]; nonfibrillar collagens enable keratinocyte and fibroblast migration and adhesion to the basement membrane [18–22]; fibronectin

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promotes aggregation of platelets and their adhesion to the damaged endothelial surface, regulates macrophage activation, enhances cell migration, and mediates ECM contraction by binding myofibroblast and collagen fibrils [24]; proteoglycans and glycosaminoglycans modulate ECM's elasticity and strength, function as signal transduction molecules, and control inflammation; matricellular proteins provide autocrine and paracrine signaling during wound healing [15–17].

Quantitative and qualitative differences in the biomechanical properties of the ECM provide variable inductive cues for local or circulating stem cells to differentiate or self-renew. The ECM modulates growth factor signaling spatially and temporally through its ability to bind ligands directly, release sequestered growth factors (reservoir), or protect them from degradation [6–14].

ECM Impairment in Chronic Wound Healing and Diabetes

Production and remodeling of the ECM are critical processes in wound healing. After injury, tissue repair and closure rely on a dynamic interaction between the ECM and local/circulating cells [6–14]. This interaction leads to a rapid onset of a temporary inflammatory respone, proliferation of fibroblasts and keratinocytes, angiogenesis, and eventually results in permanent functional healing. Chronic wounds lack a functional ECM and this condition stalls the healing process [11, 27–34]. The presence of a dysfunctional ECM in chronic wounds suggests the need for appropriate therapeutic strategies to restore structural and biochemical signals required for healing and successful tissue regeneration: restoration of the native ECM enables stimulation of the healing response and successful wound closure [11, 27–34].

High concentrations of proteases in the wound niche of diabetic patients induce degradation of ECM components: as a consequence the delicate balance regulating ECM biologic effects is disrupted [11, 27–34]. Recent studies suggest that ECM changes occurring before injuries predispose patients to chronic repair processes [11, 27–34]. For example, diabetes-induced biochemical abnormalities of the ECM maintain persistent/chronic inflammation [35, 36]. In diabetic wounds, collagen is glycosylated and its deposition in wounds is decreased; several other proteins of the ECM are glycated in diabetes, reducing the ability of cells to adhere to them [35, 36].

Cells of diabetic patients are also abnormal [37–39]: for example, fibroblasts produce less collagen and pro-angiogenic factors (such as vascular endothelial growth factor, VEGF), and lack of surface receptors for fibronectin binding (which facilitates their migration). Absence of a functional ECM in chronic wounds limits migration and proliferation of keratinocytes at wound (needed for successful reepithelialization) and of endothelial cells at the budding tips of capillaries (needed for neoangiogenesis). The ECM also controls wound contraction and scar formation by modulating the activity of myofibroblasts.

Matrix metalloproteinases (MMPs), normally responsible for ECM degradation/remodeling during healing, are also dis-regulated in diabetic wounds [40, 41]. Increased levels of MMPs (such as MMP-9) are associated with poor wound healing, decreased keratinocyte migration, and epithelial regeneration. Downregulation of other MMPs (MMP-2 and MMP-14 are absent in diabetic wounds) also contribute to the development of an impaired ECM characterized by high level of inflammation, decreased angiogenesis, and reepithelialization. In addition, the high levels of MMPs and the imbalance with their inhibitors results in an abnormal loss of both the ECM and the ECM-bound signaling molecules/growth factors.

Diabetic wounds exhibit abnormalities in growth factor expression and their interaction with the ECM is also disrupted [35, 36, 42].

Overall dysfunction of the ECM in chronic (diabetic) wounds is multifaceted; wounds fail to heal due to the presence of an imbalanced ECM and an environment hostile to cell replication/migration.

Therapeutic Potential of Bioactive ECM Scaffolds

Harnessing the biological properties of the ECM in directing reparative cell processes is essential to develop effective therapeutic strategies for nonhealing wounds. Due to its intrinsic capacity to influence and regulate cellular activity in wound healing, the ECM is an attractive therapeutic target/tool [6–14, 35, 36]. The extensive disruption of the ECM in diabetic wounds emphasizes the need for a treatment that can promote healing by supplementing, replacing, or modulating the disrupted native ECM (Fig. 9.1). Interactions between tissue-engineered bioactive ECM scaffolds and cells responsible for skin repair and regeneration (fibroblasts, keratinocytes, endothelial cells, and inflammatory cells) have been investigated in preclinical and clinical studies [43–49]. These findings suggest that healing is facilitated primarily by the application of bioactive ECM. Consistently, single cytokine/drug therapeutic approaches have shown limited clinical efficacy in the treatment of chronic wounds since wounds that are unable to rebuild a functional ECM cannot benefit from the application of growth factors or other therapies [50, 51].

Application of a structurally intact and biochemically functional ECM induces healing in chronic wounds [43–49]. The bio-inductive mechanism by which ECM scaffolds promote structural and functional repair has been described as "*constructive remodeling*." This biologic phenomenon more closely resembles regenerative than reparative processes,

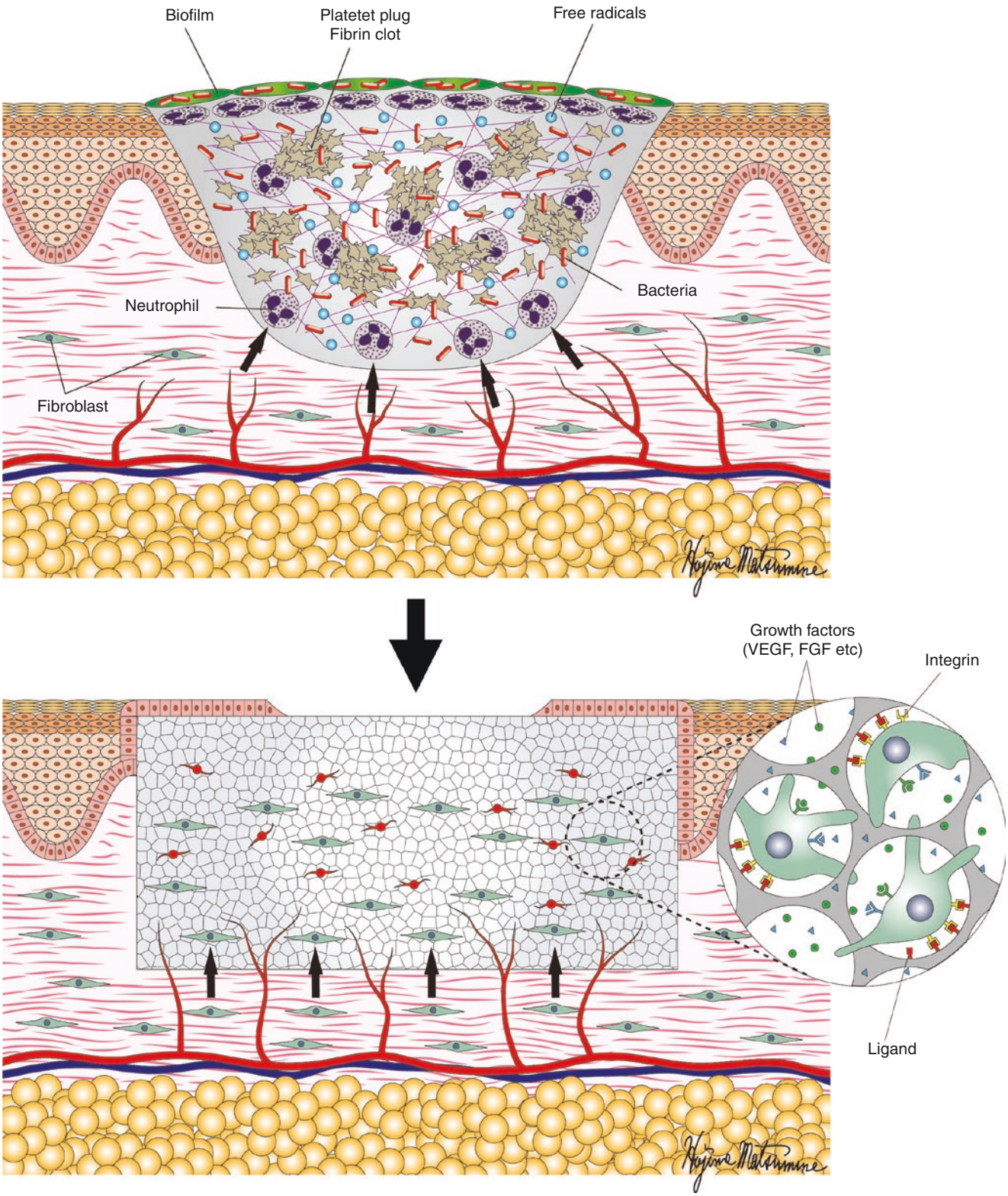

Fig. 9.1 The role of dermal matrices in wounds. (Above) Chronic wound arrested in the inflammatory phase of healing. Cellular infiltrate is depicted by the presence of neutrophils, primary cause of a toxic environment. The wound contains bacteria, free radicals, cytotoxic enzymes, and inflammatory mediators that prevent efficient healing. The wound does not pass to the proliferative phase; thus, the fibroblast cannot secrete new extracellular matrix, nor can the vessels infiltrate. In these conditions, epithelialization is not possible. (Below) Dermal matrices are acting as biological modulators, which provide the extracellular matrix with cell ingrowth and allow angiogenesis to better remove the toxic products of degradation; also, it may provide signaling for a phase shift towards healing. Dermal matrix facilitates cell ingrowth by providing ligands for cells to attach and infiltrate the matrix (close-up). Also, dermal matrix can contain or protect growth factors that can modulate the process of healing (see text for reference). VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor (With permission from: Mihail Climov, Lauren Bayer, Andrea Moscoso, et al., The Role of Dermal Matrices in Treating Inflammatory and Diabetic Wounds, Plastic and Reconstructive Surgery: September 2016, Volume 138, Issue 3S, p 148S–157S)

being in contrast to the default tissue healing response, which would be characterized by formation of scars and loss of tissue function. Instead, tissue-engineered bioactive ECM scaffolds provide appropriate signals to guide cellular repopulation and endothelial revascularization throughout a tridimensional structure [43–49]. By doing so these scaffolds are primed for intrinsic regeneration and lead to wound closure with normal, physiologic tissue formation. Today, clinical use of tissue-engineered bioactive ECM scaffolds in impaired wound healing represents the standard of care: bioactive ECM scaffolds used either alone or in combinatory therapies unlock the chronicity of impaired healing, invert persistent inflammation, and promote wound closure.

Bioactive ECM Scaffolds

Definition and General Characteristics

Bioactive ECM scaffolds are revascularized by host tissue, allow cell ingrowth and over time are degraded and replaced with host biomolecules to form structural and functional tissues [43–49]. We will focus our discussion on bioactive semisynthetic scaffolds and decellularized scaffolds, briefly mentioning also wound matrices and biological dressings such as materials derived from placental tissues. Not all biomaterials can be considered bioactive scaffolds. Bioactive scaffolds enable wound healing by providing structural, mechanical, and chemical support to regenerative processes [43–49, 52–55]: specific physicochemical properties, including chemical composition, surface tension, cross-link density porosity, and biodegradation rate, are essential to achieve these functions. Even if wound matrices and biological dressings cannot be defined as scaffolds they have been heavily promoted as such. In contrast, they fulfill only few reparative basic functions, such as reduction of the bacterial burden and control fluid loss, or delivery of high concentrations of growth factors: however, these products do not replace the lost ECM or actively contribute to optimal ECM regeneration.

Since the structural and biochemical properties of the native ECM is extraordinarily complex, it is very challenging to recapitulate its exact architecture in a synthetically manufactured scaffold. A better knowledge of the structure, biological activity, and composition of the ECM at the ultrastructural level has helped defining biological principles that can assist the design of advanced bioactive ECM scaffolds. Manufacturing methods and strategies play a critical role in determining clinical effectiveness of a scaffold. Bioactive ECM scaffolds are typically designed to serve as a temporary structure for ECM regeneration and then degrade: the kinetic of the degradation process is also a key factor controlling the release of embedded signaling molecules/growth factors and the regeneration of the ECM.

Some other clinically adopted bioactive scaffolds are derived from natural ECM (human cadaveric donors or animal sources): in these cases, the biologic properties of native ECM (such as promotion of epidermal migration, modulation of cell proliferation and mobilization, induction of differentiation of stem and progenitor cells, regulation of angiogenesis, and control of inflammation) can be retained while eliminating immunogenic components by using specific methods such as tissue decellularization.

Biological Properties of Synthetic Bioactive ECM Scaffolds

Studies that started in the early 1970s in the Fibers and Polymers Laboratory at Massachusetts Institute of Technology have shown that the adult mammal can be induced to regenerate skin that has been accidentally lost or excised. In every case it had been established previously that the excised adult skin does not regenerate spontaneously but closes by contraction and scar formation. Skin regeneration can however be partially induced with the aid of certain insoluble substrates (scaffolds) that hold a bioactive regenerative potential in the absence of pre-seeded cells. Regenerated skin is histologically and functionally different from scar and identical to physiological skin in almost all respects, including a physiological epidermis, well-formed basement membrane, well-formed capillary loops at the rete ridges of the dermal-epidermal junction, nerve endings with confirmed tactile and heat-cold feeling, and a physiological dermis. Early versions of the dermis regeneration template (DRT) lacked certain organelles (hair follicles, sweat glands, etc.) but later studies by Steve Boyce and coworkers solved this deficiency [56].

No more than three distinct processes are involved in the repair (closure) of an anatomically well-defined defect (dermis-free defect) in skin wounds: contraction originating from the edges of the defect, scar formation by stromal fibroblasts (followed by epithelialization of scar), and regeneration. The characteristic elements of the adult healing response (contraction or scar synthesis, or both) must be controlled in order for induced regeneration to occur. Extensive evidence has been presented that allows direct comparison between the relative importance of these three healing processes both in untreated standard wounds in skin and peripheral wounds as well as in wounds treated with a bioactive scaffold, the dermis regeneration template (DRT). Such comparison leads to a fairly clear description both of the mechanism of healing by contraction and scar in untreated wounds and in wounds that heal by regeneration [56].

Untreated, normally healing wounds have been studied as standardized models of wound healing in animals. Skin wounds were full-thickness wounds in several species while peripheral nerve (PN) wounds were complete transections of the rat sciatic nerve. Left untreated, these wounds normally

close by contraction and scar formation; no regeneration has been observed. The relative importance of wound closure by contraction and scar varies widely among species and has been quantitatively tabulated for several species in skin wounds [56]. Briefly, skin wounds in rodents close primarily by contraction of the mobile integument while in species in which skin is more firmly attached to subcutaneous tissues (e.g., swine, human) closure occurs to a large extent by scar. In these untreated skin and PN wounds it was observed that scar formation was blocked whenever contraction was blocked but the reverse was not observed. It was concluded that scar formation is a derivative process to contraction. These observations contradict the commonly held view that scar formation can be used to explain why adults heal their wounds spontaneously by repair, rather than by regeneration. Considering that the available processes for wound closure are just three, namely, contraction, scar, and regeneration, and that scar formation is derivative to contraction, it follows that wound closure by regeneration in adults is primarily thwarted by contraction, not by scar formation [56].

There are two sets of independent data that describe an antagonistic relation between wound contraction and regeneration. The first set of data is based on observations with injury models in various species which heal spontaneously by regeneration. These injury models include spontaneously healing skin wounds in the developing frog, the rabbit ear, the oral mucosa of the adult human, and even the axolotl, the exemplary model of perfect regeneration. In all of these examples of spontaneous healing, regeneration occurred in the virtual absence of wound contraction [56]. A second set of data was obtained in studies with a DRT, which is well known as an effective blocker of wound contraction. Collagen scaffolds can be prepared in homologous series of closely matched members, with well-defined changes in their structural features, that can be used as internal controls of a DRT. These scaffolds make ideal probes of the mechanism of induced regeneration. A few of them, including DRTs, are particularly useful as reactants that block contraction while also inducing regeneration. Over several years of study with animal models, it has been observed that wound contraction in skin [57–60] and peripheral nerves [61] was almost completely blocked when a collagen scaffold with highly specific structure was in contact with the wound. No such powerful blockade of contraction was observed when other collagen scaffolds, even slightly differing in structure, were employed. Scar formation was also blocked when such appropriately structured collagen scaffolds that regenerated skin or peripheral nerves were applied to these wounds. Although the available data do not suffice to show a causal relation between contraction blocking and regeneration, the original premise of an antagonistic relation between the two healing processes appears to be supported firmly by the data.

The molecular biological mechanism of the regenerative activity of DRT has been shown to be a dramatic modification of the contractile phenotype of myofibroblasts (MFB), the contractile fibroblasts that have been consistently implicated in wound contraction. Phenotype modification in DRT-treated wounds has been observed as a significant reduction in MFB density, dispersion of MFB assemblies, and randomization of orientation of long MFB axes. Each of these changes is associated with a significant reduction of the macroscopic contraction force that normally closes skin wounds by tensile deformation in the plane of the epidermis. PN wounds, e.g., a nerve stump resulting from transection, normally closes by application of a circumferential compressive force directed along the radial direction of the transected nerve [62].

Phenotype modification of MFB occurs following extensive contact with DRT surfaces. Such contact is facilitated by specific binding of MFB integrins $\alpha1\beta1$ and $\alpha2\beta1$ onto ligands, such as GFOFER, that are naturally present on the surface of the collagen scaffold [63]. This recent finding elucidates the mechanism of regenerative activity of DRT and highlights its sharp difference from other biomaterials.

To develop a convincing summary of the existing information on the mechanism of regenerative activity of DRT we recall the existence of at least three critical structural features of DRT that have been observed. These features reach critical levels at which maximum blocking of wound contraction coincides with incidence of regeneration. We will refer to these critical values as optimal. The optimal pore size for DRT is in the range 20–125 μm [64] while the optimal degradation half-life for this scaffold is 14 ± 7 days [61]. Insufficient data are available to identify optimal values for ligand densities for integrins $\alpha1\beta1$ and $\alpha2\beta1$. The limited evidence suggests a guideline for ligand densities that exceed 200 μM $\alpha1\beta1$ or $\alpha2\beta1$ ligands [62, 63]. Substantial modification of any of the first two structural features, and most probably of the third as well, deactivates DRT almost completely. We focus on the surface chemistry of DRT as a heretofore unknown requirement for regenerative activity; such a feature is partly or totally absent from a large number of scaffolds based on synthetic polymers that have been introduced as potential substitutes for DRT.

The combined data from skin and nerve studies support the somewhat unusual view of biological activity (expressed as cell phenotype change) that resides not in a soluble substance, e.g., an enzyme, but on a (temporarily) insoluble surface. This novel paradigm of surface biology explains the available evidence and allows description of a mechanistic pathway for DRT regenerative activity, as follows. During wound healing in the presence of DRT, myofibroblasts migrate into the porous scaffold and bind ligands present on the scaffold surface. The pore diameter is required to be large enough for cell migration but small enough to maximize the specific surface in order to account for binding of almost all

of the MFB present in the wound. Binding on the scaffold surface requires the presence of specific ligands for MFB integrins, as described above. Finally, the scaffold is required to maintain its state of insolubility, assuring that cell-scaffold binding will occur, over a period that is long enough to ensure that MFB differentiation has been consummated and that the appropriate binding machinery is already in place; further, the state of scaffold insolubility must be short enough to ensure that MFB apoptosis has not taken place and that specific binding can still occur.

An additional effect of DRT during healing is the significant reduction of MFB density which has been hypothetically attributed to nonspecific high-affinity binding of TGFβ1 on the scaffold surface. Such binding has been observed in vitro [62].

In summary, we have described the regenerative activity of DRT in terms of three critical structural features. These features appear responsible for a pathway of events that explains the data. The mechanistic events account for the change in MFB phenotype which is responsible for the blocking of contraction and the onset of regeneration of skin and peripheral nerves.

Types of Bioactive ECM Scaffolds

Bioactive ECM scaffolds currently available for clinical use and FDA-approved include engineered semisynthetic scaffolds and decellularized scaffolds [43–49, 52–55]. The former are produced from one or more specific natural materials (such as collagen, fibronectin, and hyaluronan). Scaffolds derived from decellularized tissues usually retain most biochemical and biomechanical properties of native ECMs [63–67]. However, they can greatly differ in their source material, preparation, and processing: these differences significantly affect their in vivo behavior and regenerative potential [63–67]. The percentage and absolute quantity of retained growth factors in decellularized scaffolds, as well as the preservation of the ECM structure, strongly depend upon the methods of decellularization [63–67]. Decellularized ECM scaffolds can be obtained from human or porcine dermis, porcine small intestinal submucosa, and other tissues. Most of these products contain a high percentage of collagen (mostly type I), fibronectin, laminin, and GAGs (such as heparin, heparan sulfate, chondroitin sulfate, and hyaluronic acid) [63–67].

Differences Between Bioactive ECM Scaffolds and Wound Matrices or Naturally Derived Dressings

Bioactive ECM scaffolds do not require pre-application or simultaneous application of cells to induce regeneration: their regenerative potential is uniquely dependant on their structural and biochemical properties. They mimic the regenerative capacity of native ECM that actively influences the behavior of cells in recipient tissues and does not necessarily require, to be effective, simultaneous presence of administered cells or other regenerative factors (growth factors or cytokines) [43–49, 52–55, 63–67].

A substantial difference exists with other products used in wound care such as cellularized wound matrices and naturally derived dressings. The first group includes all those matrices that do not hold an intrinsic regenerative potential but act as tridimensional delivery vectors of donor cells (keratinocytes, fibroblasts, etc.) to recipient wounds. The reparative capacity of these product depends on the biological effect of delivered cells more than on their own structure [43–49, 52–55]. Given the essential functions of ECMs in wound healing most of these products have shown inconsistent outcomes once translated in a clinical setting, possibly due to the lack of an adequate infrastructure that could enable and optimize the function of transplanted cells. Similarly, wound matrices made of synthetic polymers (for example PLA-PGA or HA-based constructs) fail to induce physiological regeneration unless combined with cells in complex tissue-engineered approaches [43–49, 52–55].

A second group of products includes naturally derived dressings that have no intrinsic bioactive properties but act as delivering methods of growth factors or regenerative cytokines [43–49, 52–55]. Similarly to wound matrices these constructs fail to recreate a native ECM, and despite their stimulatory effect on healing, they have shown limited regenerative capacity. Placental membranes are an example of naturally derived dressings: they deliver a large variety of factors promoting wound healing but do not function as dermal scaffolds for ECM replacement [68–71].

Bioactive ECM Scaffolds Adopted in the Treatment of Diabetic Wounds

Semisynthetic Collagen-Based Bioactive ECM Scaffolds

The Dermal Regeneration Template® (DRT)

Integra Dermis Regeneration Template® has been available on the market since 1996: it has been premarket approved and 510(k) cleared by the Food and Drug Administration (FDA) [72–75]. Before the development of the actual commercialized product, the first clinical application of the scaffold occurred at the Massachusetts General Hospital (Boston, MA) and the Shriners Burns Institute for the treatment of burn-related injuries. At that time the scaffold was manufactured at the Massachusetts Institute of Technology (Cambridge, MA) for the specific needs of each patient. In a multicenter study 841 severe burn sites in 216 patients were treated with the DRT showing a mean take of

76.2% (median 98%), and a mean take of the thin epidermal autograft of 87.5% (median 95%) [76]. In 1996, the FDA approved the Integra Dermal Regeneration Template® (DRT, described briefly earlier), as an urgent treatment modality for patients suffering from severe burns. Since that time, DRT has been approved by regulatory agencies in several other countries [72–75].

The template has been extensively used in clinical cases throughout the world for reconstruction of wounds caused by trauma, burns, surgery, and chronic disease showing both safety and effectiveness [72–76]. Use of the DRT minimizes the need for other surgical operations such as the use of local flaps or free tissue transfer and this has led to a substantial revision of the classic "reconstructive ladder" principles embraced by reconstructive surgeons [72–75]. We estimate that at least 600 cases, including pediatric patients, have been reported in literature as clinical studies using this scaffold [72–75]. In 2002, the FDA approved DRT for reconstructive surgery of scars. The efficacy of DRT for the induced regeneration and treatment of chronic skin wounds has also been proved, and modified versions of this device have been designed specifically to achieve this purpose [72–75].

A discrete number of clinical studies has investigated the effectiveness of the DRT in the treatment of chronic wounds and specifically diabetic foot ulcers [64, 77–80]. A recent study on 30 diabetic patients who underwent surgical debridement of diabetic foot wounds followed by grafting with DRT reported an 86.7% healing rate and a significantly more distal level of amputation [78]. Another retrospective analysis of 105 patients with diabetic foot ulcers receiving dermal regeneration template for lower extremity salvage [80] confirmed these findings: however the efficacy of DRT seems to be more limited in patients at high risk for foot amputation [80]. In another study of chronic wounds, 111 patients were treated with the DRT achieving final wound closure [81]. Other case reports have observed that the DRT leads to a skin with elasticity and mechanical properties comparable to that of normal skin but this finding have been inconsistent and would benefit from a quantitative investigation.

Recently, a prospective randomized study conducted by Driver et al. in 2015 on 307 patients demonstrated that the DRT provided 1.59-fold better healing than the standard of care [82]. This Foot Ulcer New Dermal Replacement Study (FUNDER) was a multicenter, randomized, controlled, parallel group clinical trial conducted under an Investigational Device Exemption. Investigators showed that complete wound closure was significantly greater with use of the DRT (51%) than control treatment (32%) at 16 weeks follow-up. The median time to complete wound closure was 43 days compared to 78 days for control subjects. The newly formed collagen was indistinguishable from normal dermal collagen and supported cell migration/reepithelialization of the wound. Overall the approach showed the capacity to improve not only wound-related outcomes but also quality of life of patients. Following the results of this study, the FDA approved the PMA Supplement for a newly designed DRT, marketed as Integra Omnigraft Dermal Regeneration Matrix, for the treatment of diabetic foot ulcers in January 2016.

The DRT has been successfully adopted for the treatment of other severe cutaneous defects, including the management of traumatic injuries with exposed bony tissues after trauma, degloving injuries, soft tissue losses following tumor excision, or ulcerative radiation injuries [72–75]. Case reports and case series have shown that the DRT can be grafted over vascularized bone creating a new effective bony coverage within 1 or 2 months [72–75]. Hair follicles have also been incorporated using micrografting techniques [83]. A similar approach has been adopted to provide effective coverage to exposed tendons after traumatic injuries or surgery [84]. Other applications of the dermal regeneration template include resurfacing of hypertrophic scars and keloids, post-surgical wounds of the head and neck after cancer resection [85, 86]. The DRT has also been used in combination with other treatments aimed to support and accelerate wound healing, such as negative pressure therapy [87, 88]. Combined use of negative pressure therapy and DRT seems to facilitate positive outcomes. The INTEGRA™ Meshed Bilayer Wound Matrix was specifically designed to be used for this purpose since it allows drainage of wound exudate and provides a flexible adherent covering for the wound surface. Adopting this approach Molnar et al. achieved a 93% scaffold take and wound closure in eight patients with complex wounds [89].

Recently the DRT has also been produced in an injectable form (commercialized as Integra Flowable) to be applied to undermined wounds.

Case Studies Using the Dermal Regeneration Template (DRT)

Chronic wounds are locked in a chronic inflammatory process that bioactive scaffolds can unlock this phenomenon guiding wounds towards effective repair, regeneration and closure. To highlight these mechanisms we report few clinical examples [90].

A patient suffering from pyoderma gangrenosum in a sternal wound after cardiothoracic surgery was successfully treated using the DRT. Adequate wound bed preparation was achieved after multiple surgical debridements and the use of negative pressure wound therapy in combination with systemic antibiotics and steroids. The DRT was then applied in combination with an antibacterial dressing and the adjuvant use of negative pressure wound therapy. The integrated approach eventually led to safe and complete wound closure with no further complications or the need for a skin graft (Fig. 9.2a, b).

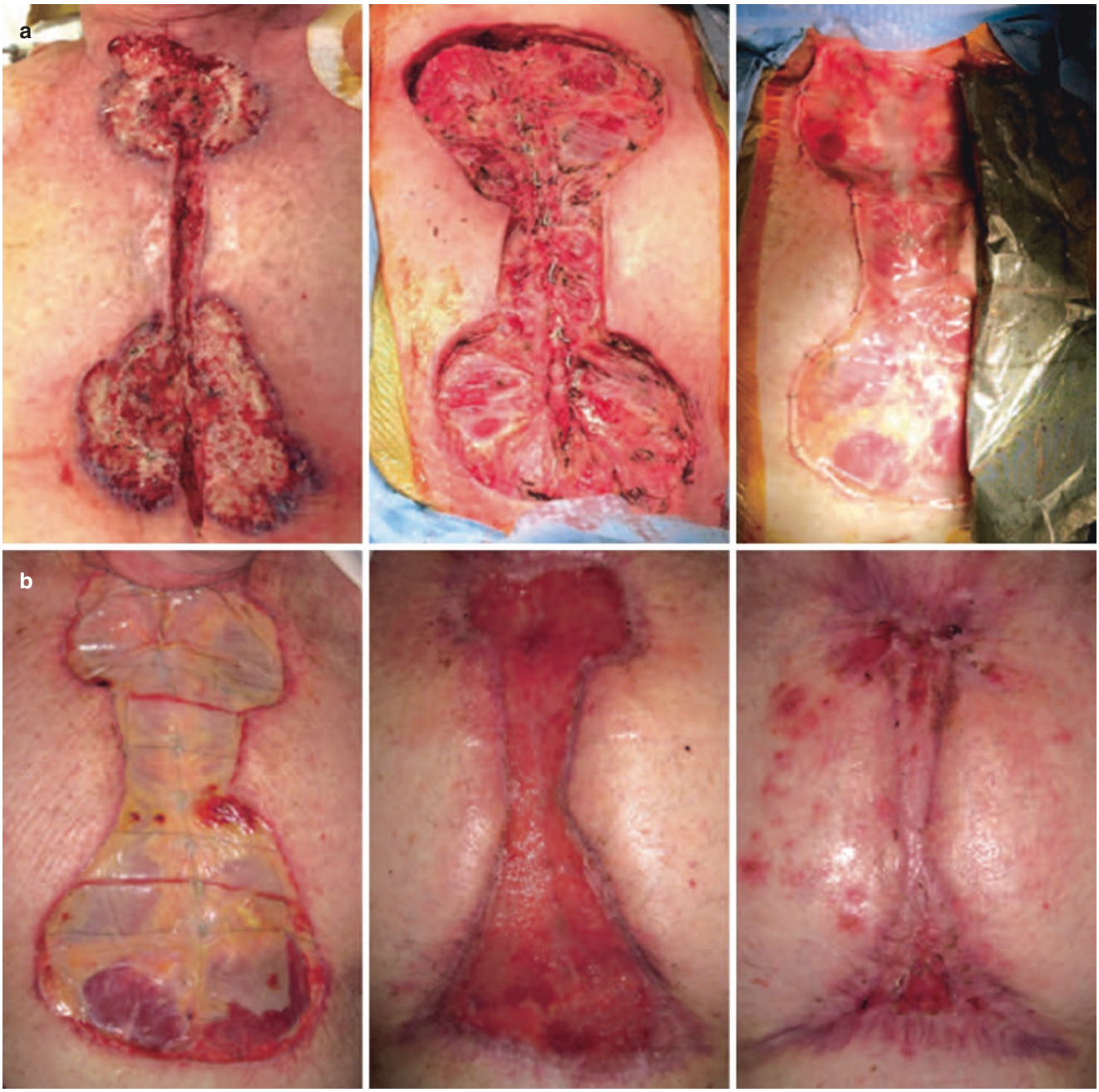

Fig. 9.2 Treatment of pyoderma gangrenosum with dermal matrix (Integra). (*Above, left*) Patient developed pyoderma gangrenosum after sternotomy. Preoperative view, final debridement. (*Above, center*) After debridement. (*Above, right*) Integra and Acticoat were placed on day 9 after debridement. (*Below, left*) Four-week appearance after Integra placement. Silicone layer is starting to peel off. (*Below, center*) Eleven weeks after Integra placement. The wound is completely granulated. (*Below, right*) Twenty-eight weeks after Integra implanting. The wound is nearly healed. (With permission from: Mihail Climov, Lauren Bayer, Andrea Moscoso, et al., The Role of Dermal Matrices in Treating Inflammatory and Diabetic Wounds, Plastic and Reconstructive Surgery: September 2016, Volume 138, Issue 3S, p 148S–157S)

Another patient with a history of sarcoidosis was affected by a chronic recurrent wound to her left ankle that had already been treated multiple times without success. An extensive debridement was performed before application of the covered the DRT in combination with an antibacterial dressing and a multilayer compression wrap. After 45 days the wound appeared ready to receive a split-thickness skin graft that eventually led to complete wound closure and healing with no further recurrence.

A young female patient affected by sickle cell disease presented a chronic lower leg wound that is unresponsive to standard treatment. After surgical debridement we chose to

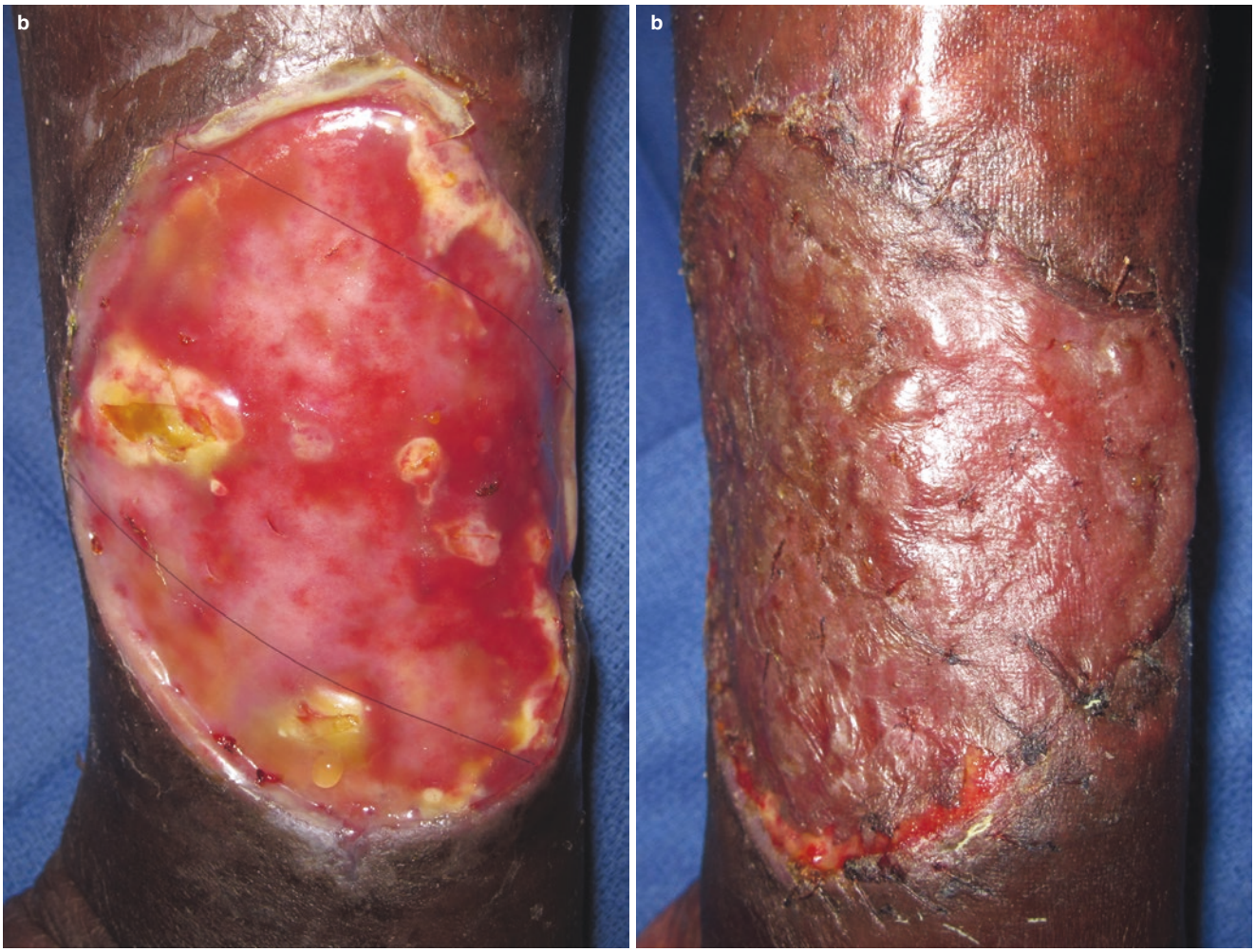

Fig. 9.3 (**a**) Sickle cell ulcer treated with dermal matrix. (Left) Sickle cell ulcer 3 weeks after Integra placement. (**b**) Two weeks after split-thickness skin graft placement (With permission from: Mihail Climov, Lauren Bayer, Andrea Moscoso, et al., The Role of Dermal Matrices in Treating Inflammatory and Diabetic Wounds, Plastic and Reconstructive Surgery: September 2016, Volume 138, Issue 3S, p 148S–157S)

synergistically combine application of the DRT with an antibacterial dressing and negative pressure wound therapy. After 30 days the treatment had led to optimal wound bed preparation: a split-thickness skin graft was performed leading to final wound closure with no further recurrences (Fig. 9.3a, b).

Other Semisynthetic Collagen-Based Bioactive ECM Scaffolds

Bioactive ECM scaffolds currently available on the market are characterized by both advantages and limitations. Although a limited number of randomized controlled trials have been performed, substantial evidence for many products is based only on small studies with variable outcome measurements and methods. Authors hope that in the future more robust randomized controlled trials comparing various products will provide better evidence in the effectiveness of each strategy for the management of chronic wounds. In particular, despite the widespread clinical use of these scaffolds only limited reports are available in literature on their use for the treatment of chronic diabetic wounds. Bioactive ECM scaffolds consist of a three-dimensional ECM, which can be of synthetic or natural origin. Most products are acellular, not immunogenic, available off-the-shelf, and can provide effective wound closure and tissue regeneration without the need of additional therapies.

Matriderm®

Matriderm® (Skin and Health Care AG, Billerbeck, Germany, not currently available in the USA) is a 1 mm-thick semisynthetic bioactive ECM scaffold made of bovine type I collagen and elastin that has shown the capacity to induce regeneration of dermis in vivo by promoting cell proliferation, cell migration, and angiogenesis [91–93]. It is derived from native bovine ECM without structural modifications (it is not cross-linked), coated with α-elastin hydrolysate and freeze-dried before use. The scaffold degrades over time, after having

induced regeneration by host cells. The product has been mostly adopted for the reconstruction of skin defects after full-thickness deep burns or chronic wounds [91–93]. Differently from other products, the presence of elastin improves the elastic mechanical properties of the scaffold. Different preclinical and clinical studies have shown similar behavior and biological properties for Matriderm® and Integra®, including the capacity to induce revascularization and lead to a scarless healing at a long-term follow-up due to a random-pattern deposition of newly formed collagen fibers [91–94]. Also, preliminary clinical trials have reported no difference between Matriderm and split-thickness skin grafts when comparing the quality of scars [95]. However, there is still a substantial lack of adequate clinical data on the use of this scaffold and most studies have been focused on the treatment of burns only.

Pelnac

Pelnac (Gunze Ltd., Medical Materials Center, Kyoto, Japan, not currently available in the USA) is a bioactive semisynthetic ECM scaffold made of collagen [96–98]. It has a bi-layered structure that consists of an external silicone layer and an internal collagen spongy layer (cross-linked atelocollagen derived from porcine tendons). Pelnac is freeze-dried and can be stored in a dry environment at room temperature for up to 3 years. It has a thickness of about 3 mm and multiple pores about 60–110 μm in diameter. It is produced in two different versions: Standard Type and Fortified With Mesh Type. The latter product has a nonadhesive silicone gauze (TREX) that provides additional mechanical reinforcement to the material. In vitro the scaffold has shown a strong capacity to promote cell proliferation, migration, and differentiation [96–98]. Clinical experience with Pelnac mostly refers to the treatment of severe full-thickness burns or post-surgical skin defects (skin tumor excision, donor site repair for flaps, lower limb reconstruction) in combination with split-thickness skin grafts [96–98]. Several studies have shown the capacity of Pelnac to promote scarless healing at a long-term follow-up [96–98]. It has also been used for the treatment of autoimmune chronic wounds. One study compared the use of Pelnac and Terudermis but clinical evidence is still preliminary and inadequate to provide a clear understanding of the differential behavior of these products [99].

Terudermis

Terudermis is another bi-layered semisynthetic bioactive ECM scaffold (not currently available in the USA) [100–102]. As previously described products it also features an external protective silicone layer, which limits bacterial contamination and controls permeability, and an internal spongy layer of lyophilized collagen (obtained from cross-linked fibrous and heat-denatured bovine collagen). Similarly to Pelnac, Matriderm, and Integra, the clinical use of Terudermis has been mostly reported in the treatment of severe burns, flap donor site defects, or post-traumatic skin injuries [100–102]. Beside clinical reports, in vitro and in vivo studies in animal models have shown that Terudermis promotes proliferation of multiple cell types known to play a key role in wound healing (such as fibroblasts and endothelial cells) leading to enhanced angiogenesis and faster wound closure [100–102]. Literature on clinical experience with Terudermis is still limited, in particular for the treatment of chronic wounds: a larger number of prospective studies will benefit the use and development of the product.

Decellularized Tissues

Human Dermis

An acellular human dermal matrix called Graftjacket (Wright Medical Technologies Inc., Arlington, TN) was developed in 2003 and first applied for the management of diabetic wounds in 2004 [103–106]. Graftjacket® is derived from processed human cadaveric dermis in which cells and telopeptides are removed while the biochemical and structural characteristics of the ECM are preserved to help stimulate healing of host tissues. The product is available in both sheet and micronized flowable (Graftjacket Xpress, a powdered human collagen version of the scaffold available for injectable use) forms with a maximum shelf life of 2 years [103–107]. In a pilot study on forty patients affected by diabetic chronic wounds and treated with either the Graftjacket or a control treatment, the Graftjacket-treated wounds showed a significantly higher wound closure (73% vs. 34%) compared to controls [103]. Another study by Martin et al. in 2005 showed complete wound closure in 14/17 patients with a mean healing time of 8.9 weeks and no complications [104]. A randomized controlled study by Brigido et al. reported a similarly higher healing rate (12/14 versus 4/14) compared to controls at a 16-week follow-up [105]. In 2008, Winters et al. reported a large, retrospective study on 100 diabetic ulcers in 75 patients treated with Graftjacket and observed complete wound closure in 91% of cases in a mean time of 13.8 weeks [106]. Similar positive outcomes were later confirmed also by other studies such as a prospective randomized multicenter trial by Reyzelman [107].

AlloDerm or AlloDerm Regenerative Tissue Matrix (LifeCell Corporation, an Acelity company, Bridgewater, NJ) is another very popular acellular bioactive ECM scaffold (likely the most widely adopted decellularized tissue in clinical practice in the United States) and was one of the first products to be developed in this field [108–113]. It was first reported in 1996 in the treatment of acute full-thickness burns [108]. It is human-derived, acellular, and available in a ready-to-use (RTU) or freeze-dried (FD) form: differences between the two forms have only minimally been compared by adequately designed clinical studies. Preclinical (in vitro and in vivo) and clinical studies have confirmed that Alloderm promotes reepithelialization, neovascularization,

and fibroblast infiltration in wounds [108–113]. Clinically, the product has been adopted in a very wide range of pathologic conditions and applications ranging from chronic wounds (including diabetic and radiation-induced), soft tissue reconstruction after trauma, abdominal wall reconstruction, and alloplastic breast reconstruction [108–113]. An injectable micronized form of AlloDerm (Cymetra) is also available [114].

Xenograft Dermis

Xenologous sources (animals) have also been investigated as sources of bioactive ECM scaffolds. Besides their biological effectiveness in promoting healing, xenologous scaffolds have shown to possess hemostatic properties and to contribute to pain reduction at the wound site through mechanisms still not completely known.

PriMatrix® (TEI Medical, Waltham, MA) is a xenogeneic ECM scaffold derived from fetal bovine dermis that is processed using ethylene oxide [115–118]. It contains Type III collagen which is abundant in fetal-embryonic scarless wound healing. PriMatrix® has shown effectiveness in the treatment of chronic diabetic wounds in several studies [115–118]. Karr et al. reported that PriMatrix® induced more rapid healing than Apligraf® in a retrospective cohort of forty patients [115]. A prospective multicenter case series performed by Kavros et al. showed complete closure of diabetic wounds in 76% of patients treated with PriMatrix® [116].

Porcine bioactive scaffolds are currently the most widely used xenologous products in wound care given the similarities between porcine and human skin. Currently available porcine-derived products include fresh, fresh frozen, lyophilized, irradiated, and aldehyde cross-linked forms. Fresh frozen preparations, lyophilized and irradiated forms require refrigeration. Aldehyde cross-linked forms lack all cellular content, leaving a sterile acellular scaffold that can be stored at room temperature. EZ Derm® (Molnlycke Health Care, US, LLC, Norcross, GA) is an aldehyde cross-linked bioactive scaffold that has been introduced clinically since the mid-1980s for the care of partial-thickness burns [119–122]. Cross-linkage increases the tensile strength of the scaffold and allows storage at room temperature for up to 18 months [119–122]. In a study involving partial-thickness burns 84.7% of patients treated with EZ Derm achieved complete wound closure, reduction in pain, fluid loss, and infection, and a minimal complication rate [119]. The scaffold also provides a moist wound environment and has an hemostatic effect: however, it does not incorporate into the wound and has to be removed at the end of treatment. Concerns about the immunogenicity of porcine scaffolds have been confirmed by studies on fresh porcine skin highlighting the presence of a type II humoral immune response likely linked to the Gal epitope: however complications have only been reported with use of fresh and fresh frozen preparations but not in products that have been cross-linked with aldehyde.

Small Intestine Submucosa (SIS)

Single-layer small intestine submucosa (SIS) of swine has been successfully adopted for the treatment of chronic wounds including venous/arterial wounds and diabetic wounds [123–128]. It is a ~0.10 mm acellular bioactive scaffold made of cross-linked collagen (predominantly types I, III, and V), proteoglycans, and glycosaminoglycans. More specifically, it is obtained from the submucosal layer of the porcine jejunum, after removal of the mucosal and muscular layers [123–128]. The collagen composition of the scaffold provides both mechanical (strength and elasticity) and regenerative properties. After decellularization to remove cellular content, the product is sterilized and lyophilized to allow long-term storage. The product is usually applied on the wound every 3–7 days until complete closure [123–128]. Recently a ~0.30 mm thick tri-layered variant has also been proposed: it is commercialized as OASIS® Ultra (Cook Biotech, Inc., West Lafayette, IN) and marketed by Smith and Nephew Inc. The higher thickness allows for higher mechanical properties, better ease of fixation, and longer durability of the scaffold. The product is characterized by a long shelf life (can be stored at room temperature) and low risk of immunological reaction. The regenerative capacity of SIS is related to its close resemblance to skin ECM and the presence of multiple growth factors [123–128]. In addition, SIS has a low porosity which allows maintenance of wound moisture. Multiple in vitro studies have shown that SIS creates an ideal micro-niche for proliferation and migration of both fibroblasts and keratinocytes [129, 130]. In addition, it has been shown that SIS plays a key role in modulating the activity of MMPs at the wound bed, reducing their inhibitory effect on wound closure, and in regulating wound inflammation [123–130]. Both single-layered and triple-layered SIS have been used clinically for the repair of chronic wounds, postsurgical defects, and traumatic injuries [123–128]. In particular, adoption of SIS in patients affected by chronic venous ulcers has shown to improve healing rates by 3–4 times compared to standard compression treatment in a prospective, randomized, investigator-blinded, controlled clinical trial: no recurrence was observed at 6 months of follow-up [123]. In another randomized, non-blinded study, SIS was compared to cellularized wound matrices in the treatment of chronic diabetic wounds: both treatments showed a remarkable capacity to induce prompt and effective wound closure but no difference was observed between the two approaches [124]. Overall SIS seems to improve the healing rate of chronic wounds heal by ~55% compared to standard treatments [123–130].

Others

Acellular scaffolds can be applied in combination with cells but this is not currently common clinical practice even if cellularized scaffolds have shown to promote rapid vascularization and wound closure. Even so, there is not sufficient clinical evidence that combination of bioactive

ECM scaffolds and cells provide better induction of wound healing. Gammagraft® (Promethean LifeSciences, Inc., Pittsburgh, PA) is a particular type of cellularized scaffold since it is actually a skin allograft that has been gamma-irradiated and that can be stored at ambient temperature with a shelf life of 2 years [131–133]. The use of Gammagraft® has been reported in several case series for the treatment of chronic venous wounds, diabetic foot ulcers, burns, and other chronic wounds [131–133]. However, evidence of their effectiveness is still limited and a larger number of prospective randomized controlled trials is required for a better analysis. Another xenologous acellular scaffold is the Matristem Wound Matrix (ACell, Inc., Columbia, Maryland) [134–136]. This lyophilized scaffold is derived from the basement membrane and subjacent lamina propria of the porcine urinary bladder and it is known in literature as urinary bladder matrix (UBM). The scaffold is available in different forms such as a single-layer sheet, multilaminate sheets, and meshed sheets. A particulate form, MatriStem MicroMatrix®, has also been produced for use in tunneled/undermined wounds [137].

Overview of Other Wound Matrices and Naturally Derived Dressings

Most bioactive ECM scaffolds are processed to be acellular and consist only of a mixture of proteins. Cellular scaffolds provide a more abundant source of cytokines and growth factors that can promote wound healing but might also elicit an immune response to their cellular content [138–141]. To avoid this problem some scaffolds incorporate fetal cells that are characterized by low immunogenicity or cultured epidermal cells that do not express major histocompatibility class II HLA-DR antigens [138–141]. However, it is important to define the difference between cellularized bioactive ECM scaffolds and cellularized wound matrices [138–141].

Complex regenerative wound matrices and naturally derived dressings have been engineered to retain the bi-layered structure found in normal skin [138–141]. This category mostly includes cellular products that require presence of cells or specific growth factors to actively promote wound healing. Despite their clinical effectiveness bi-layered wound matrices (in particular those containing cells) are often difficult to manufacture and store (very limited shelf life), and expensive [138–141].

Permaderm and Tissuetech contain living keratinocytes and fibroblasts [142, 143]. Studies have shown that the presence of these cells leads to a high angiogenic effect, early revascularization, and good modulation of wound inflammation.

Apligraf® (Organogenesis, Canton, MA) consists of an external layer of neonatal human foreskin keratinocytes and an internal layer of bovine-derived collagen matrix seeded with neonatal human foreskin fibroblasts (NHFFs) [144–149]. The product provides both cells and matrix to promote healing. Apligraf was the first engineered skin to be FDA approved for use on chronic wounds (1998: venous wounds; 2000: diabetic wounds) [144–149]. It has been successfully used to treat acute wounds, chronic wounds, and burns [144–149]. In preclinical and clinical studies it has shown the ability to deliver cytokines (Interferon-α, Interferon-β, IL-1, IL-6, and IL-8), growth factors (e.g., PGDF), and ECM components to the wound bed [144–149]. FDA approval for use on chronic diabetic wounds was granted based on the results of a randomized trial involving 208 patients from 24 centers in the USA [144]. Apligraf led to complete wound closure at a 12-week follow-up 56% of wounds compared with 38% in the control group, reducing incidence of osteomyelitis and amputations. Falanga et al. also demonstrated in a multicenter randomized trial involving 293 patients with chronic venous wounds that Apligraf and compression therapy were more effective in inducing healing than compression therapy alone[145]: a subsequent study from Sabolinski et al. confirmed these outcomes [146].

Dermagraft (Advanced BioHealing, La Jolla, CA, USA) is the other living skin equivalent (together with Apligraf) approved by the FDA for treatment of chronic diabetic wounds [148–150]. It contains fibroblasts attached to an absorbable substrate [148–150]. In a multicenter, controlled single blind study involving 281 patients, Dermagraft-treated wounds showed an higher rate of wound closure compared to control-treated wounds (38.5% vs. 31.7%) [148]. The same effectiveness of dermagraft in the management of chronic diabetic wounds has been shown my multiple other studies [149, 150].

OrCel is another products that incorporates fibroblasts and keratinocytes into a lyophilized collagen scaffold [151–153]. The matrix has been mostly used for the management of burns [151]. In a clinical trial on 120 patients OrCel showed effectiveness in inducing closure of chronic venous wounds (59% vs. 36% of controls) [152].

Tissuetech has shown outcomes similar to those obtained by the use of Apligraf in the management of chronic diabetic wounds (wound closure rate: 65–91%) in both observational studies, randomized controlled trials and retrospective reviews [154–156].

Placental Membranes

Placental membranes have been used for effective treatment of wounds for over 100 years (first reports in literature date back to 1910). Placental membranes have been mostly used for the treatment of burns, and only occasionally for the management of chronic wounds [69, 157–161]. Placental

membranes are known to be a very rich source of growth factors and cells including mesenchymal stem cells (MSCs), neonatal fibroblasts, and epithelial cells [69, 157–161]. The low immunogenicity of placental membranes permits their allogeneic use [69, 157–161]. Despite these products have been referred to in the literature as scaffolds, there is growing evidence that they mostly act as wound matrices and naturally derived dressings. Their regenerative capacities are not related to their ECM structure but rather to their content of cells, growth factors, and cytokines (delivery device) [69, 157–161]. All these properties synergistically interact to promote and accelerate wound healing, tissue regeneration, and repair: placental membranes have strong anti-inflammatory, antimicrobial, anti-scarring, and pro-angiogenic properties [69, 157–161]. Different products have been developed and delivered to the market for clinical application. Use of fresh tissue has been limited due to its short shelf life and the risk of disease transmission [69, 157–161]. Placental membranes can also be cryopreserved or dehydrated for better preservation; however, dehydrated products have shown lower (up to 7.5-fold) angiogenic, anti-inflammatory, and antioxidant effects [69, 157–161].

Currently, there are more than 25 commercial placental products, all of which are devitalized. Placental membranes are regulated as human cells, tissues, or cellular or tissue-based products (HCT/P) under the 21 CFR part 1271, Section 361 of the Public Health Services (PHS) Act [69, 157–161]. As other allografts, they do not require premarket approval, allowing for a faster regulatory pathway to the market. Very limited peer-reviewed case studies utilizing commercially available placental membranes have been reported in the literature and mostly relate to only two products: EpiFix® (MiMedx Group, Marietta, GA) and Grafix (Osiris Therapeutics, Inc.). EpiFix is a dehydrated amniochorion, also called dHACM [68, 162–164]. In a smaller study 3 patients affected by chronic diabetic wounds were treated with EpiFix: two of them (66.7%) reached complete wound closure within 5.5 weeks after treatment [68]. There have been also three prospective, randomized, controlled trials using EpiFix to treat chronic diabetic wounds: two single center and one multicenter [162–164]. In one study (total of 25 patients) EpiFix lead to complete closure 92% of wounds after 6 weeks compared to 8% in the control group [162]. These findings were substantially confirmed in the other two studies. In one of these 95% of patients in the EpiFix group achieved complete wound closure versus 35% of those in the control group [163]. Even if encouraging, the results of these studies are still preliminary and should be confirmed in a multicenter study with a larger number of patients. Grafix Prime® (Osiris Therapeutics, Inc., Columbia, MD) is a product designed to preserve the native components of the human placental membrane in a cryopreserved form that can be used on demand at the point-of-care; it must be stored at −80 °C before use and has a 2-year shelf life [165–167]. It is the only commercially available placental membrane product to contain viable cells together with Core® chorionic mesenchyme (Osiris Therapeutics, Inc., Columbia, MD). Grafix has been used for the treatment of acute and chronic wounds (including diabetic wounds), or burns [165–167]. In a recent multicenter randomized controlled trial Grafix has shown to improve the healing rate of chronic diabetic wounds compared to standard of care and to reduce complications associated to the treatment [165]. The study reported an overall healing rate of 62% at a 12-week follow-up. In addition, the probability of closure was 67.8% among patients who crossed over from the control group to Grafix after failing to heal with control treatment. These findings confirm previous outcomes obtained by the use of the same product in the treatment of venous ulcers [166]. In another retrospective single-center study on chronic wounds of various etiologies Grafix demonstrated a 76.1% wound closure rate at 12 weeks follow-up with no adverse events [167]. However, the study is limited by the lack of a proper control group.

In summary, placental membranes have shown promising results as wound matrices/naturally derived dressings in the treatment of chronic diabetic wounds and seem to hold some of the properties required to promote healing of complex wounds. However, lack of an effective regenerative ECM is a major limitation of these products. Further studies are required to confirm early reports. The number of commercially available products is growing rapidly placing a priority on the need for accurate scientific and clinical data to support their use.

Discussion and Conclusions

Future Directions in the Development and Optimization of Bioactive ECM Scaffolds

Clinicians desire a cost-effective, topically applied, bioactive ECM scaffold that can promote prompt, complete skin regeneration without complications. The evolution of bioactive ECM scaffolds will depend upon a greater understanding of the role played by individual ECM components in the regenerative niche of uninjured/injured tissues. Significant advances and important milestones have been achieved in the last decades: today tissue engineering keeps moving forward at a rapid pace towards this ideal goal. One of the major trends in the field has been the gradual, constant shift from the use of human/animal tissues to the adoption of semisynthetic products. Human/animal-derived products have a theoretical potential for disease transmission and immune reactions; furthermore, decellularized bioactive ECM scaffolds are limited by size, shape, degradation rate, physical form, or mechanical strength, and availability. On the other hand, products using only synthetic components have shown an inadequate biological effectiveness.

The ideal standard seems to be represented by the use of hybrid semisynthetic bioactive ECM scaffolds made of both synthetic and purified biological components with customizable, tunable biological activity. Besides their biochemical and structural regenerative potential, as described in this chapter, these materials have the potential to be used as platforms for cell-based therapy, controlled release drug delivery, and incorporation of sensor technology. In particular, increasing focus has been placed over the last years towards the development of advanced bioactive ECM scaffolds [168–170]. These might provide extra features such as enhanced regenerative properties (embedded growth factors), control of hemostasis, delivery of local analgesia, or control of infections. By "functionalizing" bioactive ECM scaffolds or tuning their physical properties, it is possible to provide treatments with optimal antibacterial, anti-inflammatory, and adhesive properties. Bioactive ECM scaffolds can also be functionalized with specific growth factor-binding sites: unfolding the exact mechanisms by which the ECM modulates growth factor activity will lead to the design of more efficient integrated ECM-growth factors therapies. For example, it has been proposed that scar-free healing can be achieved through the addition of TGF-β to bioactive ECM scaffolds [171]. Other growth factors, such as epithelial growth factor or FGF have also been proposed as candidates for embedment in advanced functionalized bioactive ECM scaffolds [172, 173]. Another example involves the use of Stromal cell-derived factor, VEGF, or PDGF to induce endothelial cell migration and vessel formation, and ultimately promote angiogenesis [174–176]. Spatially and temporally controlled sequential release of growth factors from a bioactive ECM scaffold can be modulated by changes in biophysical properties of the ECM such as its density, porosity, charge, and hydrophobicity. Embedding of antibiotic particles or analgesics in bioactive ECM scaffolds is also being investigated. Finally, development of the next generation of biomimetic/bioactive ECM scaffolds will also incorporate growing knowledge on the use of cells and stem cells in tissue repair and regeneration: bioactive ECM scaffolds will become more and more an integrated regenerative tool capable of delivering ECM biochemical/biomechanical cues, growth factors, regenerative particles and (stem) cells to enhance healing potential through their synergistic ability to recreate all aspects of a regenerative niche.

Conflict of Interest: I.V.Y. has participated in the founding of Integra LifeSciences, Plainsboro, NJ. He currently has no financial connection with the company and owns no stock of Integra LifeSciences.

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Microvascular Changes in the Diabetic Foot

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Abstract

Diabetes affects the microcirculation through many different pathological mechanisms, including endothelial dysfunction and abnormal neurovascular control. These functional changes in microvascular function have a compounding relationship with structural changes in the cutaneous microcirculation of the diabetic foot. Ultimately, such adverse adaptations in function and structure contribute to the formation of diabetic foot complications such as ulceration, and in more severe circumstances to amputation. Indeed, diabetes and its associated complications place an enormous economic burden on public health systems, globally, highlighting the need for early intervention and prevention. In recent decades, several noninvasive imaging techniques and tests of microvascular reactivity have evolved that may have the potential to allow clinicians to more accurately predict the risk of foot ulceration in those with diabetes, as well as provide the ability to monitor wound healing rates and determine the success of therapeutic interventions. This chapter will summarize these methods used to assess the cutaneous microcirculation while also describing the respective roles of hyperglycemia, insulin resistance, and inflammation in endothelial dysfunction and its complex relationship with neurovascular function.

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Introduction

The concept of diabetic microangiopathy emerged during the first half of the twentieth century when the description of its three major elements, neuropathy, retinopathy, and nephropathy, were associated with diseased arterioles, capillaries, and venules [1]. In parallel, observations that diabetic foot ulcerations (DFUs) could develop despite the presence of peripheral pulses have also highlighted the central role of the microcirculation in the pathophysiology of such complications. The involvement of an occlusive "small-vessel disease" was supported by studies of amputation specimens describing endothelial proliferation in diabetic patients, responsible for arteriolar occlusion. Although occlusive microvascular disease has been subsequently overturned and other factors such as neuropathy, abnormal pressure loading, and susceptibility to infections have been recognized as major risk factors for DFUs, impaired cutaneous microcirculation remains the ultimate cause of necrosis in the diabetic foot [2].

In recent decades, numerous structural and functional abnormalities of the cutaneous microcirculation have been observed in diabetes, revealing the diversity of the pathological processes that affect the microcirculation over the time course of the disease across different capillary beds. This is why the term "diabetic microangiopathy" does not refer to something uniform [3], and that complexity may explain why the mechanisms leading to impaired microvascular function and whether such dysfunction has a direct or indirect role in the onset and healing of DFUs have only been partly elucidated. Ultimately, a better understanding of the specificities of microvascular changes in the diabetic foot and their close interactions with diabetic peripheral neuropathy (DPN) is essential to developing new treatments for this unmet clinical need. Finally, whether cutaneous microvascular function can be used as a reliable biomarker that could predict wound healing is another important issue that requires further examination.

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The Cutaneous Microcirculation

In recent years the cutaneous microcirculation has emerged as a unique index of systemic microvascular function and pathology-induced microvascular dysfunction due mostly to its superior accessibility. However, given that the microcirculation is responsible for mediating adequate nutrient and gas exchange between blood and tissue, many clinical and exploratory studies of the diabetic foot and its associated complications (e.g., diabetic foot ulcers) also use microvascular assessment to directly determine changes in cutaneous tissue health over time.

Anatomy of the Skin Microcirculation

The microcirculation consists of resistance vessels such as small arteries, arterioles, and venules that typically range from 10 to 300 μm in diameter and capillaries (≈6 μm) [4]. In the cutaneous microcirculation, these small arteries, arterioles, and venules collectively form two horizontal plexuses in the dermis. The upper plexus in the papillary dermis, from which nutritive capillary loops arise (Fig. 10.1), is connected by ascending arterioles and descending venules to a lower network located at the dermal-hypodermal interface [5]. Arteriovenous anastomoses (AVAs) are direct connections between the arterial and venous networks.

Each blood vessel has three distinct layers defined as the outer tunica adventitia, the central tunica media, and the inner tunica intima. The adventitia's proportion of the vascular wall is variable dependent on the vascular bed and is comprised of elastin, collagen, fibroblasts, mast cells, and macrophages [6]. Compared to other tissues, the adventitia of blood vessels within the cutaneous microcirculation also present a high density of sensory, sympathetic, and parasympathetic nerve axons that do not penetrate the media [7]. However, these nerve fibers do pass close to the media, which is comprised predominantly by vascular smooth muscle (VSM) cells, demonstrating the major influence of autonomous neural control in cutaneous microvascular function [8].

As blood vessels decrease in diameter, the proportion of the vessel wall occupied by VSM cells remains similar due to a decrease in the number of VSM cell layers to as little as one layer in the arterioles. However, the actual volume fraction of VSM cells within the media typically increases to 70–85% of the total media volume demonstrating an increased ability of microcirculatory blood vessels to control vessel diameter and subsequently maintain optimal cutaneous perfusion [6]. Deep to the media and an internal elastic lamina is the intima consisting of a layer of endothelial cells that forms a continuous internal cover of the vascular wall. The endothelium, which sometimes forms ridges that project into the lumen, also frequently projects through fenestrations of the internal elastic lamina, regularly making contact with VSM cells of the media [6]. This important anatomical feature allows for an interaction between the endothelium and VSM cells that is crucial to maintaining normal vascular tone.

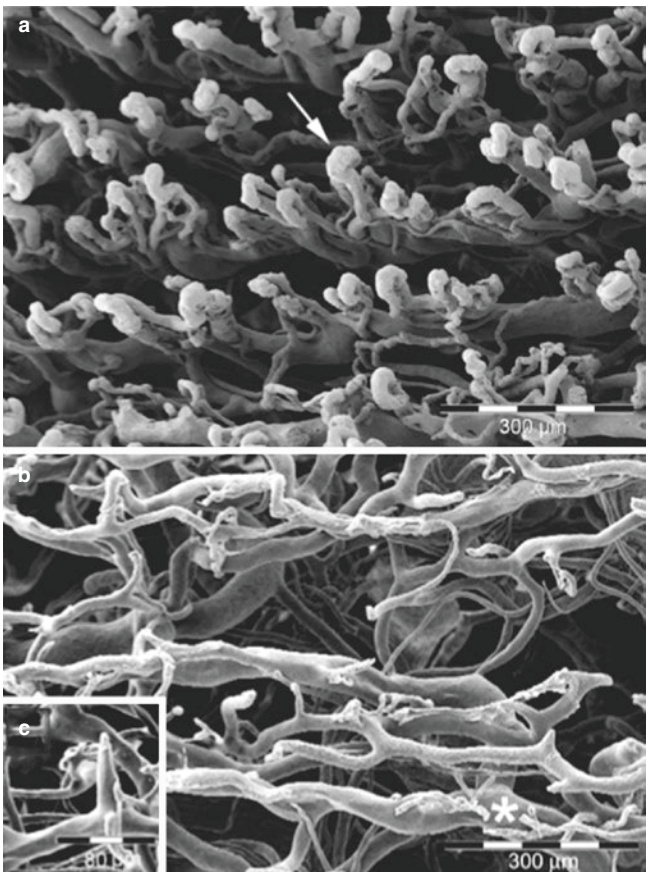

Fig. 10.1 Organization of the skin microcirculation in the toe. In healthy subjects, capillary loops arise from the upper plexus (**a**), while in diabetes the nutritive microvasculature is damaged (**b**, high magnification **c**). From ref. [119], with permission

Physiology of the Cutaneous Microcirculation

In addition to neural control, vascular tone is modulated by shear stress, metabolic mechanisms and the arteriolar myogenic response [9]. Shear stress, the force exerted on the endothelial wall by vascular blood flow, is considered the predominant regulator of vasomotion [10]. Together, with other agonists such as insulin, acetylcholine, adenosine triphosphate, adenosine, bradykinin, and histamine (Fig. 10.2), shear stress acts on the endothelium to stimulate the synthesis of several vasodilating (nitric oxide [NO], prostacyclin [PGI_2], and endothelium-derived hyperpolarizing factors) and vasoconstricting (endothelin-1, angiotensin II, prostanoids, such as thromboxane A_2, and isoprostanes) substances that are released to the VSM, mediating widening and narrowing

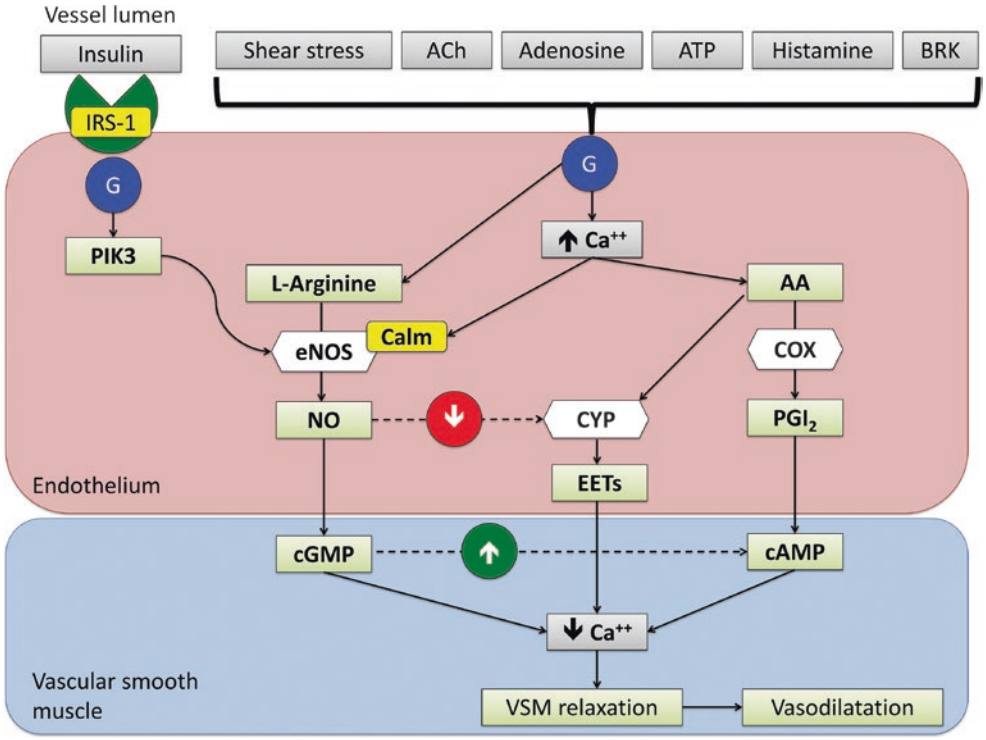

Fig. 10.2 The interaction between the three main vasodilatory pathways (NO, PGI_2, and EETs) in normal healthy vascular function. Adapted from (Loader J, et al.; in Diabetes and Exercise, Springer, 2017). *ACh* acetylcholine, *ATP* adenosine triphosphate, *BRK* bradykinin, *IRS-1* insulin receptor substrate-1, *G* G-protein phospholipase, *PIK3* phosphatidylinositol 3-kinase, Ca^{++} free intracellular calcium, *eNOS* endothelial nitric oxide synthase, *NO* nitric oxide, *CYP* cytochrome metabolites, *EETs* epoxyeicosatrienoic acids, *AA* arachidonic acid, *COX* cyclooxygenase, *Calm* calmodulin, PGI_2 prostacyclin, *cGMP* cyclic guanosine monophosphate, *cAMP* cyclic adenosine monophosphate, *cGMP* cyclic guanosine monophosphate, *VSM* vascular smooth muscle, ↑ upregulates, ↓ downregulates

of the blood vessel, respectively [10, 11]. The balance of this mechanism is not only critical to maintaining normal vascular tone, but also essential in promoting optimal microvascular and cutaneous health through the regulation of pro-inflammatory cytokines, leukocyte recruitment, platelet aggregation and adhesion, angiogenesis and VSM cell proliferation [10].

Given its status in those with diabetes, the vasoactive role of insulin is important to consider when exploring underlying mechanisms of cutaneous microvascular disease. A key component of insulin's metabolic action is its ability to dilate resistance vessels and precapillary arterioles to increase total blood flow and the microvascular exchange surface perfused within the skeletal muscle, respectively [12]. Thus, allowing for optimal postprandial-nutrient delivery to the most peripheral vascular beds such as those of the cutaneous microcirculation. Unique to other agonists, insulin achieves its vasodilatory role by synthesizing NO exclusively via a calcium-independent pathway [13]. In brief, circulating insulin signals the insulin receptor of the endothelial cell, activating G protein-phospholipase interactions that stimulate the phosphatidylinositol 3-kinase pathway [13, 14]. This cascade of signaling activates protein kinase B to phosphorylate and activate endothelial NO synthase (eNOS), which ultimately synthesizes NO from the amino acid, L-arginine [14, 15]. Further to its vasodilatory action, insulin also simultaneously induces vasoconstrictive mechanisms via mitogen-activated protein kinase that stimulates the secretion of endothelin-1 [12].

Shear stress and agonists of vasomotion (such as acetylcholine, adenosine triphosphate, adenosine, bradykinin, and histamine) also stimulate NO synthesis via the phosphatidylinositol 3-kinase, calcium-independent pathway [10]. However, unlike insulin, these agonists also stimulate the synthesis of both NO via a calcium-dependent pathway and PGI_2. In brief, agonist-mediated G protein-phospholipase interactions deplete the endothelial cell calcium concentration, inducing a calcium influx via store-operated channels and potassium channel activity [16]. Free intracellular calcium then binds to calmodulin, activating eNOS to synthesize NO, and liberates arachidonic acid, initiating the cyclooxygenase pathway to synthesize PGI_2 [16, 17]. Once synthesized in the endothelial cell, NO and PGI_2 diffuse to the VSM cell where they increase the formation of cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP), respectively. The actions of cGMP and cAMP are identical, mediating a reduction in intracellular calcium concentration that induces VSM relaxation. Noting that cGMP also promotes cAMP activity to

increase the overall sensitivity of the mechanism demonstrates the synergistic influence of NO and PGI_2 to modulate cutaneous microvascular tone [10].

In healthy conditions, NO and PGI_2 are the dominant mediators of microvascular dilation. However, endothelium-derived hyperpolarizing factors (EDHFs), which include epoxyeicosatrienoic acids (EETs), also contribute to inducing vasodilation [8]. In brief, arachidonic acids that are liberated by an increase in endothelial intracellular calcium concentrations also stimulate several cytochromes to synthesize EETs in the endothelium [18]. Diffusion of EETs to the VSM cell induces hyperpolarization and subsequent VSM relaxation via opening of potassium channels and closure of calcium channels that causes a decrease in VSM intracellular calcium concentrations. Indeed, normal NO bioavailability suppresses cytochrome activity and the subsequent synthesis of EETs. However, when NO activity is disrupted, EETs production and its vasodilatory influence may increase in order to maintain normal vascular function [18]. Although these dynamic systems with multiple regulators allow for vasodilation and vasoconstriction to still occur normally even in the event of weakening of a vasoactive pathway, a significant dysfunction within a central mechanism, such as that occurring in diabetes, may still substantially impair overall vascular function [10].

Methods to Explore the Cutaneous Microcirculation in the Diabetic Foot

Recent technological advances have allowed researchers to perform noninvasive assessment of cutaneous microcirculatory health in specific regions of the foot with improved accuracy, a development that is of considerable importance given that global measurement of microvascular perfusion may not reflect regional deficits observed in those with diabetes [19].

Laser Doppler

One of the most common methods adopted by researchers over recent decades to quantify changes in cutaneous microvascular function has been laser Doppler flowmetry (LDF). The laser Doppler principle is based upon the phenomenon that when a laser beam emitted by the imaging device hits moving red blood cells in the cutaneous vessels, the light undergoes a change in wavelength (Doppler shift) and the backscatter is detected by the device [20]. The laser Doppler signal, quantified as the product of mean red blood cell velocity and concentration, provides an index of cutaneous perfusion referred to as *flux*, rather than a direct measure of cutaneous blood flow [21]. Using a single-point laser probe and a high sampling frequency of approximately 32 Hz, LDF is capable of accurately quantifying rapid variations in cutaneous blood flow within a volume of 1 mm^3 or smaller. However, considering the anatomical heterogeneity of the cutaneous microcirculation and the relatively small vascular region that can be assessed, LDF is subject to increased spatial variability and thus presents relatively poor reproducibility between measurements [21].

Laser Doppler imaging (LDI) is an alternative laser Doppler-based imaging technology that scans a tissue bed of interest (e.g., the volar surface of the forearm) to produce a 2D image and map cutaneous blood flux within that region, with each pixel representing a separate perfusion value [21]. In contrast to LDF, where the laser unit is in direct contact with the skin, LDI emits a laser beam at a set distance above the skin surface. Therefore, given that LDI is capable of assessing a large area of the cutaneous microvasculature in a single scan, the spatial variability associated with LDF is reduced. However, the image rate of LDI is much slower than that of LDF and therefore it is not possible to detect rapid changes in cutaneous perfusion. Furthermore, research commonly performs a single scan to acquire baseline and post-intervention perfusion values, resulting in images that correspond to a brief time-point during the assessment of microvascular function. Consequently, critical events (e.g., peak responses to tests of vascular reactivity) may be completely missed, introducing temporal variability and severely limiting the reproducibility and interpretation of LDI data.

Capillaroscopy

Whereas laser Doppler provide an index of general cutaneous perfusion, capillaroscopy allows for researchers to noninvasively perform direct in vivo assessment of the density, recruitment, and blood flow velocity of the capillaries [21], the normal function of which are critical to maintaining adequate gas and nutrient exchange between the microcirculation and the tissues to promote optimal tissue health. Using a microscope with epi-illumination and imaging systems, capillaroscopy is often performed at the periungual region where nailfold capillary loops are oriented parallel to the skin, imaging the width of a few millimeters [22]. As capillaroscopy visualizes erythrocytes, rather than providing an image of the capillary wall, only capillary loops with circulating erythrocytes at the time of assessment will be captured [23]. For a nailfold capillary pattern to be considered normal, capillary loops ranging from 6 to 15 μm in diameter should be homogenously distributed [23]. Although nailfold capillaroscopy has been shown to have diagnostic applications in diseases that affect the digital cutaneous microcirculation, capillaroscopy outside this periungual region has not been found to have clinical applications. Indeed, capillary loops in these other cutaneous regions are oriented perpendicular to the skin and, thus, visualization of capillary perfusion is limited to the top of the loop, only providing an index for the density of functioning capillaries per region of interest.

Transcutaneous Oxygen Tension

Given that oxygen is vital to maintaining optimal tissue health and promoting wound healing processes, assessing the oxygenation in the cutaneous microcirculation may be considered as an important index of skin blood perfusion. Transcutaneous oxygen tension ($TcPO_2$) is an established technique that allows for noninvasive evaluation of the partial pressure of oxygen in cutaneous tissue. Correlating well with peripheral arterial disease, $TcPO_2$ may also have value in predicting healing rates in those suffering from DFU and amputation rates in those with peripheral arterial disease or ischemic ulcers [24]. In brief, using a probe that is applied to the surface of the skin and heated to 45 °C in order to induce vasodilation, $TcPO_2$ measures the transfer of oxygen molecules from the blood vessels to the skin surface with a decreased $TcPO_2$ reading indicating decreased oxygenation [24]. Given that $TcPO_2$ only assesses the area of tissue directly under the probe, it may be more clinically relevant to perform multiple measurements across varied regions rather than conducting a single assessment. Indeed, a regional perfusion index, calculated by dividing the foot $TcPO_2$ value by a baseline $TcPO_2$ value measured at the chest, may provide more reliable data [19]. It must be noted that $TcPO_2$ may be less reliable in warm ambient environments and in those who are active smokers, have autonomic neuropathy or vascular calcification, with or without peripheral arterial disease; or in those who have an active infection, edema, or callus, due to arteriolar shunting that causes $TcPO_2$ readings to be less representative of the true state of microvascular health [19, 25]. The "oxygen challenge," in which patients are administered 100% oxygen during the $TcPO_2$ assessment, has been proposed as a strategy to more accurately detect true values that represent peripheral artery diseases in such conditions [19].

Near-Infrared Spectroscopy

Cutaneous oxygen concentration can also be noninvasively assessed using near-infrared spectroscopy, a technique that has traditionally been the most popular method of estimating or measuring tissue oxygenation [26]. Near-infrared spectroscopy, which may also provide an indirect method of evaluating mitochondrial function [27], uses near-infrared light emitted from a probe placed on the skin and is based on the principles that specific wavelengths of red and near-infrared light have the ability to penetrate through biological tissue; absorption of these specific red and near-infrared wavelengths are dominated by hemoglobin; and absorption varies between oxygenated and deoxygenated hemoglobin [28]. Light emitted by the probes typically penetrates the tissue to a depth of 2 cm and is detected by photodetectors, which can provide estimations of total hemoglobin, oxyhemoglobin, deoxyhemoglobin, and tissue oxygen saturation [28].

Traditionally, evaluation of DFU and monitoring of healing rates has been based on surface assessments that involve a clinician manually measuring the length and width of the wound; a method that may be limited by the fact that irregular wound shapes may lead to inaccurate estimations of size and unfavorable recommendations for wound treatment [29]. The diffuse photon density wave methodology of near-infrared spectroscopy allows for measurement of oxyhemoglobin and deoxyhemoglobin at depths of up to ~3 cm, and, thus, may provide more clinically valuable information about the wound on a subcutaneous level (e.g., revascularization) and a more advanced method of evaluating the evolution of DFU [30]. The efficacy of diffuse near-infrared spectroscopy was evaluated in a recent study that monitored the progression of human DFU longitudinally over 24 weeks, finding that the technique had an 82% predictive value for DFU outcomes within 4 weeks of wound monitoring [29].

Structural Changes in the Microcirculation of the Diabetic Foot

Structural abnormalities of the arterioles were observed in the mid-twentieth century in the retina and the kidneys. Arteriolar hyalinization, corresponding to the thickening of the walls of arterioles from amputated diabetic limbs, was also described [1]. Such arteriolar remodeling was confirmed decades later in patients with type 2 diabetes, who had systemic structural alterations of subcutaneous small resistance arteries, as indicated by an increased media-to-lumen ratio. These abnormalities are characterized by hypertrophic remodeling and are associated with impaired endothelium-dependent vasodilation in vitro. Of note, they affect patients with and without hypertension [31]. Another structural abnormality, that has been inconsistently reported, is the decrease in skin capillary density that was observed in the lower limb muscle of patients with diabetes when compared to controls [32]. Additionally, endoneurial capillary density was also found to be reduced in diabetic patients with neuropathy [33]. However, in skin biopsies from the dorsum of the foot of patients with type 1 diabetes, no decrease in vessel density was observed when compared to age-matched controls, and density was not related to complications of diabetes [34]. Aside from density, abnormal morphology of cutaneous capillaries in the dorsum of the foot such as capillary enlargement, a sign of hypoxia, has also been reported [2].

One of the most notable structural changes of the microvasculature in diabetes involves thickening of the capillary basement membrane. These abnormalities are more pronounced in the leg, likely because of the higher hydrostatic pressure and the inability of the skin microvasculature of diabetic patients to respond adequately to postural changes [35]. Interestingly, improved glycemic control with intensive insulin therapy in patients with type 1 diabetes contributed

to decreasing the width of the skeletal-muscle capillary basement membrane in parallel to a decrease in HbA1c [36]. Capillary basement membrane thickness in the nerve and the skin of the lower limb correlates with the extent of neuropathy in diabetes [33]. Such thickening of the basement membrane may affect oxygen and nutrient exchanges. Moreover, it may also limit the compensatory arteriolar dilation in response to reduced perfusion pressure [2]. Despite these deleterious adaptations, structural microcirculatory changes are not likely to have a primary role in the pathophysiology of DFUs; rather, they potentiate the functional impairment that affects different parts of the cutaneous microcirculation, most prominently, the arterioles and AVAs.

Cutaneous Microvascular Dysfunction in the Diabetic Foot

In those with diabetes, there is a loss of ability for the cutaneous microcirculation to adequately respond to stimuli, as reflected by disrupted thermoregulation. Within the thermoneutral zone, the AVAs play a major role in thermoregulation. However during a heat challenge, more of the superficial circulation vasodilates to dissipate the body heat with the latter accounting for approximately 90% of total skin blood flow [37]. Due to impaired microvascular reactivity of cutaneous arterioles, patients with type 1 or type 2 diabetes show altered heat dissipation during exercise [38, 39]. Although cutaneous microvascular dysfunction may be systemic, the following will focus on specificities of the diabetic foot, concluding with a comparison to the upper limb.

Endothelium-Dependent Microvascular Reactivity

In vitro, isolated vessels from subcutaneous resistance arteries of patients with type 1 diabetes had a decreased relaxation response to the administration of acetylcholine (ACh), suggesting impaired endothelial function [40]. This was further confirmed in vivo by using venous occlusion strain-gauge plethysmography, showing an association between chronic hyperglycemia and impaired endothelium-dependent vasodilation in patients with type 1 and type 2 diabetes [41, 42]. As technologies used to assess skin blood flow only quantify a relative signal, they are usually performed in conjunction with tests of vascular reactivity to provide an index of vascular health and insight into underlying mechanisms of vascular function. Post-occlusive reactive hyperemia (PORH) is an index of endothelium-dependent vasodilation at the precapillary level in the cutaneous microcirculation. In the foot of patients with type 1 diabetes capillary recruitment measured with intravital video-microscopy during PORH is blunted. This is related to an increase in apparent capillary density at rest (capillaries that are spontaneously perfused), which suggests that capillaries are already recruited maximally in the feet of diabetic patients [43].

Local heating is an easy-to-perform test of vascular reactivity that allows for exploration of both neurovascular-dependent (initial peak) and endothelium-dependent vasodilation (delayed plateau). Early studies that measured delayed hyperemia after a heating challenge reported impaired cutaneous vasodilation on the foot of patients with type 1 diabetes, compared with controls. Moreover, the vasodilatory response was also negatively correlated with the duration of diabetes [44]. Although the underlying physiological pathways of local heating were not known at that time, this study provides evidence of endothelial dysfunction in diabetic patients. These results were later confirmed by other studies in children/adolescents with type 1 diabetes [45], in patients with type 2 diabetes [46], and in patients with elevated fasting plasma glucose concentrations, at risk of developing type 2 diabetes [47]. Another study observed a decreased plateau of hyperemia in response to local heating on the foot of patients with diabetes and neuropathy, compared to diabetic patients without neuropathy, that is associated with reduced expression of eNOS in the cutaneous microcirculation [48]. However, the presence of an ulcer was not associated with any further impairment of local thermal hyperemia [49]. Other studies, focusing on the early response to local heating, have provided evidence supporting the role of abnormal neurovascular function in cutaneous microvascular dysfunction in those with diabetes (developed below).

One of the most commonly utilized methods of exploring endothelial function in the cutaneous microcirculation is transdermal iontophoresis of ACh coupled with laser Doppler. In the foot of patients with type 1 or type 2 diabetes and neuropathy, the response to iontophoresis of ACh measured with LDI was decreased in comparison to that in healthy controls. Interestingly, this abnormal response is observed both in the presence or the absence of peripheral vascular disease [48]. These findings were confirmed in another study that compared the change in skin blood flux assessed with LDF in response to ACh iontophoresis on the dorsum of the foot versus the forearm in 52 patients with type 2 diabetes. Among them, those who had neuropathy had significantly decreased microvascular reactivity [50]. Indeed, such abnormal vasodilation during iontophoresis of ACh seems to occur early in the pathophysiology of the diabetes as it has been shown to be abnormal in patients at risk of developing type 2 diabetes and in those with type 2 diabetes at low risk of developing an ulcer [51, 52]. Similarly, abnormal reactivity was observed in 56 young patients (aged 9–22) with type 1 diabetes in whom ACh-induced vasodilation measured with LDF was negatively correlated with diabetes duration and HbA1c levels [45]. In addition

to ACh, iontophoresis of sodium nitroprusside is often performed to assess endothelium-independent vasodilation with many studies also reporting abnormal responses in diabetes. However, noting that iontophoresis of sodium nitroprusside is a technique that is limited by current-induced vasodilation, which itself is a significant confounder, interpretation of this data should be approached with caution.

Although there are a significant number of studies that have established endothelial impairment in the cutaneous microcirculation of the diabetic foot, few have controlled other potential confounders known to influence endothelial function such as age, cardiovascular disease, or drugs affecting the microcirculation. Finally, the nature of the aforementioned tests (i.e., local heating and iontophoresis) that involve both the endothelium and sensory nerves limits the interpretation of the data due to the difficulty in discriminating endothelial dysfunction from abnormal neurovascular control.

Neurovascular Function

The ability of the skin to adequately regulate blood flow in response to temperature variations or to a variety of mechanical and chemical stimuli is highly dependent on the existence of intact neurovascular function. Indeed, diabetes is also associated with nerve dysfunction that contributes to impaired reactivity of the cutaneous microvasculature and has long been observed through disturbances in cold and heat pain thresholds [53]. There are different components of the nervous system that are involved in cutaneous microvascular reactivity. The neurogenic vascular response is one of them, commonly (improperly?) referred to as the "axon reflex", and is dependent on capsaicin-sensitive primary afferent nociceptive neurons. These neurons co-express sensory transducers like the transient receptor potential vanilloid-1 (TRPV1), as well as vasodilatory neuropeptides, including substance P and calcitonin gene-related peptide (CGRP) [54, 55]. Therefore, these dual sensory-efferent functions would occur at the same nerve ending and would not involve any axonal conduction. While the majority of nerves in skin are sensory, autonomic nerves are also abundant. In particular, the skin vasculature and sweat glands receive dual autonomic innervation by sympathetic noradrenergic and sympathetic cholinergic fibers [56]. While cutaneous postganglionic autonomic nerves contain classical neurotransmitters such as norepinephrine or ACh, they also release co-transmitters like CGRP, Neuropeptide Y, or vasoactive intestinal polypeptide (VIP) [56].

Primary Sensory Nerves

In patients with type 1 diabetes and microvascular disease, small C-fiber dysfunction is detected, even when clinical neuropathy is not. Indeed, patients who present microalbuminuria or retinopathy had abnormal early responses to local heating measured with LDI on the dorsum of the foot compared to patients without microvascular disease. Moreover, it was negatively correlated with HbA1c levels, suggesting that sensory nerve dysfunction is related to glycemic control [57]. Transdermal iontophoresis also partly depends on intact functional sensory nerves when blood flux is measured at a small distance from the iontophoresis site. In diabetic patients without neuropathy and in healthy controls, local anesthesia decreases hyperemia at the foot and at the forearm levels. In patients with DPN however, local anesthesia reduced hyperemia on the forearm but has no effect on the dorsum of the foot, suggesting that abnormal sensory nerve-dependent microvascular reactivity primarily affects the lower limb [58]. Such sensory nerve-dependent vasodilation on the dorsum of the foot also showed a high sensitivity to detect DPN progression during a 3-year follow-up of a cohort of diabetic patients [59].

Sympathetic Diabetic Neuropathy

Patients with diabetes both with and without clinical neuropathy have demonstrated impaired thermoregulation [60]. In those with uncomplicated type 2 diabetes (without any comorbidities), vasodilation in response to whole-body heating is impaired, suggesting abnormal cholinergic sympathetic function and/or impaired cholinergic cotransmission (possibly involving Substance P) [61]. At rest, impaired noradrenergic sympathetic tone translates into increased cutaneous vascular conductance compared with controls [61], most probably due to altered function of AVAs. Indeed, vasoconstriction of AVAs is mostly under control of noradrenergic tone and sympathetic neuropathy may result in increased opening of the shunt, thus deviating blood flow from the arteriolar to the venular bed through a low resistance, high velocity vascular network. Despite this, skin sympathetic nerve activity recorded via microneurography of the peroneal nerve during whole-body cooling was not impaired in patients compared with matched healthy participants. Concomitantly, cold-induced reflex vasoconstriction was similar in the two groups [62].

In summary, microvascular reactivity related to capsaicin-sensitive primary afferent nociceptive neurons is impaired, predominantly in the lower limb, in those with type 1 or type 2 diabetes. Such dysfunction seems to precede overt clinical neuropathy; however, results are conflicting due, in part, to the relatively small sample size of the studies and the heterogeneity in the criteria used to define DPN. Sympathetic neuropathy is also frequent and affects arterioles and AVAs, the latter being responsible for higher resting cutaneous vascular conductance in diabetes. This dysfunctional phase precedes organic structural damage and progressive decrease in intraepidermal nerve density [63].

Table 10.1 Differences in skin microvascular function between the upper and lower extremity in type 1 and type 2 diabetes

	Type 1		Type 2	
	Upper limb	Lower limb	Upper limb	Lower limb
Endothelium-dependent				
LTH plateau	↓	↓*	↓	↓
PORH	?	↓↓	↓	–
Ionto ACh direct	?	↓↓*	↓*	↓↓*
Neurovascular				
LTH pic	↓	↓	↓	↓
Ionto ACh indirect	↓*	↓↓*	↓*	↓↓*

? Conflicting results; * in the presence of DPN, microvascular reactivity was further impaired

Differences Between Upper and Lower Extremity

Many studies that have explored cutaneous microvascular function on the forearm of diabetic patients have demonstrated systemic microvascular dysfunction in both type 1 and type 2 diabetes (Table 10.1). In a cohort of 181 adolescents and young adults with type 1 diabetes and 96 age-, race-, and sex-matched controls, local thermal hyperemia recorded with LDF was decreased in those with diabetes, suggesting early impairment of endothelial function. Importantly, the strongest predictor of the microvascular response to local heating was HbA1c, highlighting the importance of blood glucose control on systemic microvascular function [64]. In contrast, there have been conflicting results regarding other tests such as iontophoresis of ACh. While several reports concluded that there is no difference between patients with type 1 diabetes and controls [65, 66], an elegant study found that differences in iontophoresis protocols (e.g., variability in the administered quantity of electrical current) may vary the conclusions [67]. The lack of standardization of the methods is therefore a major issue, while differences in study populations are another possible explanation. Indeed, comorbidities, duration of diabetes, medications, and differences between control populations can all influence microvascular reactivity tests, making cross-study comparisons difficult. For example, the use of low-dose aspirin is very common in diabetic patients and may interfere with iontophoresis of ACh, which is at least partly dependent on prostanoids [68]. For this reason, it may be necessary to combine different functional reactivity tests, as well as other biomarkers.

In patients with type 2 diabetes, however, the change in skin blood flux in response to iontophoresis of ACh on the forearm is decreased compared to age-matched controls [69]. These results were later confirmed in patients with type 2 diabetes with and without vascular complications, using a variety of reactivity tests such as post-occlusive reactive hyperemia, local heating, or iontophoresis of both ACh and sodium nitroprusside [70]. In parallel, markers of endothelial dysfunction such as plasminogen activator-1 (PAI-1) and tissue plasminogen activator (tPA) were increased in all patients. In contrast, von Willebrand factor (vWF), free tissue factor pathway inhibitor (f-TFPI), and the soluble form of thrombomodulin (s-TM) were only increased in patients with vascular complications [70]. Similarly, pulse wave velocity, a marker of arterial stiffness, was increased in patients with clinical micro- and/or macrovascular disease only, suggesting that functional alterations of the endothelium precede structural changes of the arterial wall, even in appropriately treated patients [70].

Differences in the cutaneous microcirculation between the upper and the lower limb may explain why diabetic ulcers develop on the foot. In healthy subjects, absolute perfusion and $TcPO_2$ of non-glabrous skin is similar between the upper and the lower limb [71]. However, the reactivity of the microvasculature expressed as the percentage change from baseline measurement in response to reactivity tests is lower in the foot than in the forearm, and this difference is consistent in diabetic patients with and without DPN [71]. In patients with type 2 diabetes, the vasodilatory response to ACh iontophoresis was more altered in the foot than in the forearm when neuropathy was present, while DPN was associated with reduced microvascular reactivity both on the foot and the forearm [50]. Similarly, local anesthesia had no effect on microvascular reactivity on the foot of patients with DPN when assessing sensory nerve-dependent vasodilation, showing further impairment in these patients [58]. Ultimately, differences between upper and lower extremities may be explained by higher hydrostatic pressure in the lower limb that leads to microvascular remodeling and a subsequent decrease in the ability to respond to stimuli. Furthermore, differences in the density of AVA between the foot and the forearm may also be an explanation [37].

Mechanisms Involved in Diabetes-Related Microvascular Dysfunction

The mechanisms underlying microvascular dysfunction in diabetes, whether endothelium-dependent or neurovascular, are complex and multifactorial, and are directly affected by factors including glycemic control, insulin resistance, obesity, and low-grade inflammation. These varied factors may explain the differences in the evolution of microvascular dysfunction over the course of the disease between type 1 and type 2 diabetes [72]. In this section we will briefly summarize the cellular and molecular mechanisms of microvascular injury in diabetes that are not specific to the skin, but are relevant to all microvascular complications.

Effects of Hyperglycemia on Endothelial Function

Since the 1960s, three classical pathways have been described to explain the mechanisms through which hyperglycemia damages the vessels, namely, aldose reductase and the activation of the polyol pathway, advanced glycation end products (AGEs), and protein kinase C activation (PKC) [73]. All of these pathways contribute to the production of ROS, such as superoxide, in the vascular wall and are also involved in nerve damage in diabetes, which itself impairs microvascular reactivity, the latter depending on intact sensory nerves [12].

Protein kinase C (PKC) is a family of ubiquitously expressed regulatory enzymes involved in cellular signal transduction. It plays a central role in several vascular functions, such as the regulation of vascular cell permeability, extracellular matrix synthesis, angiogenesis, and regulation of vascular smooth muscle contractility [74]. Increased lipid diacylglycerol (DAG) causes a sustained activation of PKC in diabetes. Moreover, hyperglycemia is responsible for increased PKC activation through upregulated transcription [75]. Such activation of endothelial PKC results in endothelium-dependent microvascular dysfunction through inhibition of the NO and EDHF pathways. Moreover, it activates the endothelin-1 pathway and enhances ROS production resulting in increased vascular tone [74]. Ruboxistaurin, an inhibitor of PKCβ, has been assessed in randomized clinical trials with interesting preliminary results in the treatment of microvascular complications, where it was found to prevent a decline in glomerular filtration rate [76], as well as decreasing the incidence of vision loss [77]. More relevant to DFUs, a recent study demonstrated that cutaneous fibroblasts from patients with type 1 diabetes exhibit elevated levels of PKCδ, associated with inhibition of insulin signaling and function, that lead to impaired wound healing [78].

Another mechanism that contributes to endothelial dysfunction involves increased polyol pathway flux. Glucose metabolism through the polyol pathway is very low in nondiabetic persons. In the presence of hyperglycemia however, glucose conversion to the polyol sorbitol by aldose-reductase is increased using NADPH as a cofactor. This may deplete cytosolic NADPH, which is necessary for regenerating reduced glutathione (GSH), a potent cellular antioxidant [79]. NADPH is also a cofactor for NOS, thus cytosolic depletion in the endothelium may decrease NOS activity. Decreased antioxidant capacity favors eNOS uncoupling, shifting NOS activity towards decreased NO production and increased superoxide ($O_2^{\cdot -}$) generation.

Hyperglycemia also induces the formation of intracellular and extracellular advanced glycation end products (AGEs) through both enzymatic and nonenzymatic reactions. In endothelial cells, AGEs alter the structure and function of intracellular and extracellular matrix proteins causing abnormal interactions with other matrix proteins and with integrins [79]. Moreover, activation of receptors for AGEs (RAGE) by extracellular AGEs leads to a signaling cascade that stimulates NADPH oxidase (NOX). This increases ROS and contributes to eNOS uncoupling. The role of NOX-derived oxidative stress in diabetic kidney disease and in DPN has been established [80]. Another target of RAGE signaling is NF-κB translocation to the nucleus, which increases the transcription of proteins including endothelin-1 and ICAM-1 and activates inflammatory pathways [81].

Generation of ROS appears to be a unifying pathway between hyperglycemia and endothelial dysfunction and a key player in endothelial cell damage (Fig. 10.3). Indeed, in addition to reducing the bioavailability of NO, superoxide rapidly reacts with NO to form peroxynitrite ($ONOO^-$), which exerts a variety of deleterious effects in endothelial cells. Peroxynitrite-mediated alterations include depletion of the eNOS cofactor tetrahydrobiopterin (BH4), which further enhances eNOS uncoupling, DNA injury, and activation of poly (ADP-ribose) polymerases (PARP) [82]. In addition to their role in DNA repair, PARP are involved in proinflammatory reactions in endothelial cells (e.g., ICAM-1 production in response to TNFα). Over-activation of PARP by peroxynitrite depletes the cell from NAD^+, therefore impairing mitochondrial electron transport leading to cell death by necrosis [73]. Neutralization of peroxynitrite or PARP inhibition have demonstrated interesting effects in the treatment of microvascular complications in various experimental models [73]. Interestingly, PARP inhibition improved wound healing in diabetic mice [83].

Mechanisms that protect against oxidative stress may also naturally occur in endothelial cells. In the skin of the lower limb, mitochondrial superoxide dismutase (SOD), which converts superoxide into H_2O_2 (accounting for EDHF-dependent vasodilation), is overexpressed in patients with recently diagnosed type 2 diabetes while the subepidermal endothelial cell area is preserved [84]. This suggests that increased SOD is an early mechanism protecting against the formation of mitochondrial ROS. In contrast, reduced levels of serum SOD have been associated with a higher incidence of microvascular complications [85–87].

Recent experimental data suggest that inhibiting the degradation of EETs, which also account for EDHF activity, prevents microalbuminuria and renal inflammation in an overweight hyperglycemic mouse model during conditions of NO deficiency [88]. Altogether, this suggests that EDHF pathways play a compensatory role in maintaining microvascular function in diabetes, when the NO-dependent pathway is impaired. Restoring the bioavailability of EETs by blocking soluble epoxide hydrolase, an enzyme involved in their degradation, has been proposed as a treatment for diabetes vascular complications [89]. Clinical trials are currently being conducted to test this hypothesis in humans.

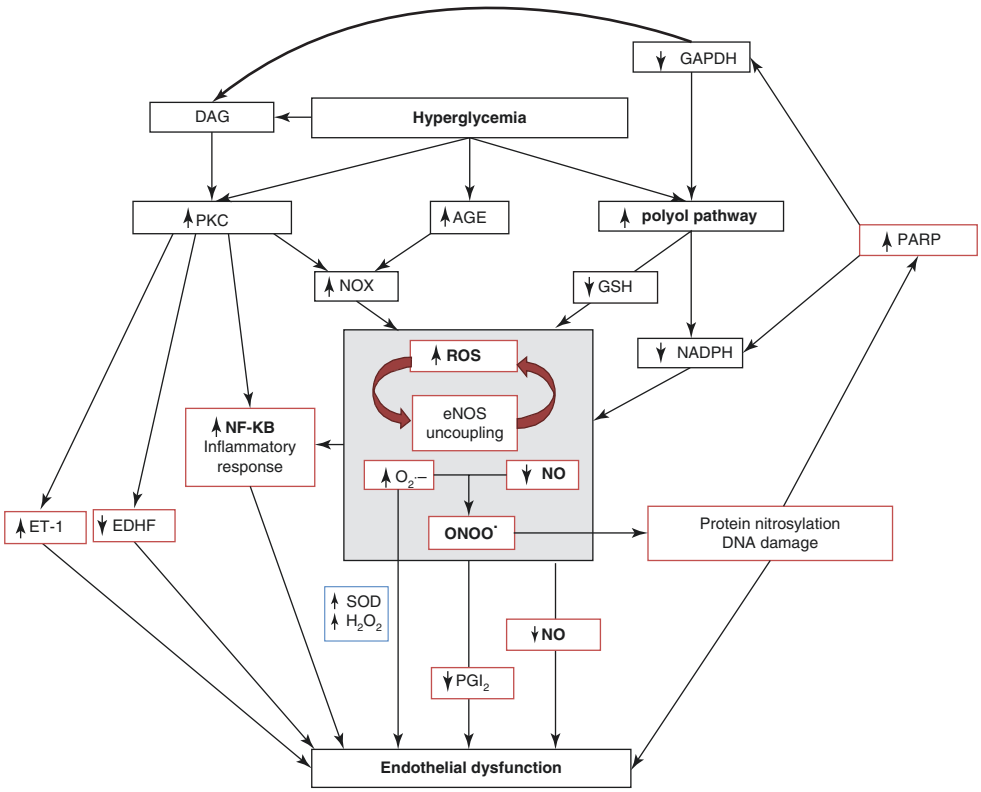

Fig. 10.3 Mechanisms linking hyperglycemia to endothelial dysfunction involve reactive oxygen species (ROS) as a key actor. Deleterious pathways appear in red, while protective mechanisms are in blue. *AGE* advanced glycation end product, *DAG* diacylglycerol, *EDHF* endothelium-derived hyperpolarizing factor, *eNOS* endothelial NO synthase, *ET-1* endothelin, *GAPDH* glyceraldehyde 3-phosphate dehydrogenase, *GSH* glutathione, *NADPH* nicotinamide adenine dinucleotide phosphate, *NF-κB* nuclear factor-kappa B, *NO* nitric oxide, *NOX* NADPH oxidase, $O_2^{\cdot-}$ superoxide, *ONOO* peroxynitrite, *PARP* Poly (ADP-ribose) polymerase, *PKC* protein kinase C, *SOD* superoxide dismutase

Role of Insulin Resistance, Obesity and Inflammation in Endothelial Dysfunction

In addition to its pivotal role in the regulation of cell metabolism, insulin has a wide range of hemodynamic effects including increased NO-dependent vasodilation and capillary recruitment [90]. In endothelial cells, insulin upregulates gene expression of eNOS, VEGF, and ET-1, and downregulates VCAM-1. Although upregulation of ET-1 does not exert beneficial effects on endothelial function, it is worth noting that it involves different signal transduction. While IRS/PI3K/Akt signaling mediates the protective effects of insulin, upregulation of ET-1 depends on the MAPK pathway [75]. Interestingly, targeted knockout of the insulin receptor in the vascular endothelium of mice led to accelerated atherosclerosis without changes in insulin levels or sensitivity, suggesting an overall beneficial effect of insulin on endothelial function, independently of its metabolic effects [91].

The ability of insulin to enhance endothelium-dependent vasodilation in the lower-limb is impaired in obese patients, with a negative correlation between leg blood flow during metacholine injection and percentage of body fat [92]. Interestingly, endothelium-dependent vasodilation is similarly reduced by 40–50% in obese patients with or without type 2 diabetes, while endothelium-independent responses remain preserved [92]. In contrast, insulin resistance in patients with type 2 diabetes has been associated with impaired endothelium-dependent and endothelium-independent function, independently from obesity, in parallel with low-grade inflammation [93]. This suggests that obesity, which plays a key role in the development of insulin resistance, potentiates the deleterious effect of insulin resistance on vascular function. Indeed, in the skin of lean subjects, elevated free fatty acids impaired capillary recruitment and ACh-mediated vasodilation, while they were improved after free fatty acids were lowered in obese subjects [94]. The main mechanism involves the binding of elevated free fatty acids to Toll-like receptors (TLR), which initiate a pro-inflammatory environment through NF-kB activation. They also activate PKC, which inhibits IRS/PI3K/Akt signaling and therefore downregulates eNOS, while the MAPK pathway is preserved. Hence, decreased NO and increased ET-1 disrupt the endothelium's ability to properly vasodilate in response to stimuli. Finally, intracellular oxidation of free fatty acids generates ROS, which amplifies the aforementioned deleterious mechanisms [95].

The immune system plays an important role in the onset of low-grade inflammation in response to insulin resistance. Recently, it was shown that mast cells were increased in the skin of diabetic patients when compared to that of controls, while mast cell degranulation correlated with biomarkers of inflammation such as IL-6 or TNFα. In parallel, macrophage polarization towards the M1, "pro-inflammatory" phenotype, was observed in the foot skin of patients with diabetes [96]. In a mouse model of a diabetic ulcer, mast cell deficiency was associated with impaired wound healing. Moreover Substance

P improved wound healing only in the presence of mast cells, suggesting that Substance P mediates its beneficial effects in wound healing, at least partially, through mast cells [96]. The role of neuropeptides and mast cells in wound healing is detailed in another chapter (Chap. 8).

Role of Neuropathy

Although diabetic neuropathy has been classically defined as a microvascular complication, the relationship between skin microvascular dysfunction and neuropathy in diabetes is complex and has not yet been fully elucidated. From a mechanistic perspective, peripheral neuropathy and endothelial dysfunction share similar pathophysiological pathways. Indeed, some of the mechanisms described above for endothelial cells are also encountered in neurons [97]. For example, increased intracellular glucose increases the polyol pathway flux. In addition to depleting the cellular NADPH reserve, increased aldose-reductase transformation of glucose leads to sorbitol accumulation, which de-differentiate Schwann cells into immature cells [98]. Oxidative stress and AGEs also play an important role in the pathophysiology of DPN [97]. It is therefore tempting to think that endothelial dysfunction and DPN are two concomitant phenomenon of the same origin.

Pioneering work using electron micrographs of sural nerve capillaries showed that endoneural microangiopathy was related to the severity of neuropathy, thus supporting a causal relationship between impaired microvasculature and diabetic neuropathy [99]. Recent experimental data provided further insight into the complex relationship between endothelial function and neuropathy, suggesting that endothelium impairment is sufficient to cause neuropathy trough the involvement of the Desert Hedgehog (Dhh) pathway [100]. This is consistent with a large observational study conducted in participants with prediabetes, diabetes with or without neuropathy, and controls, demonstrating that endothelial function was a strong, independent predictor of DPN. It further suggested that endothelial dysfunction mediates the deleterious effects of diabetes on cardiovascular risk and DPN [101].

A unifying hypothesis could involve neuronal sensors that are also present at the surface of endothelial cells. In recent years, growing evidence has suggested that transient receptor potential vanilloid subfamily member 1 (TRPV1) may play a key role in vascular health and metabolism, with possible involvement in the pathogeny of diabetes [102]. Other sensors may be involved. Indeed, pressure-induced vasodilation, an early microvascular response that delays the decrease in cutaneous blood flow produced by local low pressure, is abnormal at the foot level diabetic patients with or without DPN [103, 104]. This reflects microvascular fragility in the skin and involves Acid-Sensing Ion Channel-3 (ASIC3), a voltage-insensitive cation channel that has been shown to be a neuronal sensor for appropriate adjustment to pressure changes in the cutaneous microcirculation [105].

Microvascular Abnormalities and the Risk of Ulcers

Is Cutaneous Microvascular Function a Predictor of Wound Healing?

Improving the ability to predict and prevent diabetic foot ulceration is imperative because of the high personal and financial costs associated with this complication. Many studies have attempted to associate microvascular complications with DFU or DFU healing. Although one of the main factors that predicts the incidence of DFU is diabetic neuropathy [106, 107], a history of other microvascular complications (retinopathy, nephropathy) have also been significantly associated with the development of ulcers in diabetes [107]. However, the role of impaired cutaneous microvascular function as a predictor of DFU and/or wound healing is still not clear. In a study conducted in 20 patients with type 2 diabetes with DFU and 20 without ulceration, compared to 18 control subjects, microvascular reactivity to local heating was not different between the two groups of patients. In that study, $TcPO_2$ was also unable to discriminate between individuals with and without ulceration [46]. However, these data sets conflict with other reports in which both $TcPO_2$ and PORH, a global marker of microvascular function, were impaired in patients with DFU when compared to diabetic patients without DFU [108]. Indeed, a recent meta-analysis of tests that predict wound healing suggests that the overall quality of available evidence is low. Although TcPO2 was the strongest predictor of wound healing and amputation, data were too limited for the efficacy of other tests to be conclusive [109]. This might be explained by the relatively low sample size of included studies, which highlights the need to further evaluate tests of microvascular reactivity as predictors of DFU and wound healing.

Effect of Revascularization on the Skin Microcirculation

Peripheral arterial disease is four to six times more prevalent in patients with diabetes between the ages of 45 and 75 years than in those without diabetes and the male-to-female ratio approaches one. According to current guidelines, vascular surgeon intervention is the primary action for achieving wound healing in patients with DFU and PAD [110], as the presence of PAD considerably slows down the healing process as a direct consequence of the limited supply of oxygen,

nutrients, and topical factors. However, even if correction of hypoxia improves cutaneous microcirculation in the neuropathic diabetic foot, the effect of successful lower extremity arterial revascularization on the impaired foot microcirculation with diabetes is not clear [111]. There is one study showing that impaired vasodilation in the diabetic neuropathic lower extremity leads to functional ischemia, which improves considerably but is not completely corrected with successful bypass grafting surgery. This could be an explanation to why patients with diabetes and neuropathy may still be at high risk for the development of foot ulceration or the failure to have an existing ulcer heal despite adequate correction of large vessel blood flow [112]. However, it is clear that the literature is lacking in this topic and more studies are needed before further conclusions are made.

Latest Developments

To date, most studies that have assessed diabetes-related microvascular dysfunction in humans have used iontophoresis coupled with laser Doppler. In addition to its tremendous variability, several methodological issues should be considered when dealing with iontophoresis. Firstly, the response to ACh iontophoresis has long been primarily attributed to a NO-dependent endothelial response, while the involvement of a COX-dependent pathway is likely [8]. Recently, ACh-mediated vasodilation in human skin has also been shown to involve EDHFs [113]. Interestingly, the relative contribution of NOS/COX to ACh-mediated vasodilation varied according to ACh concentration and duration of infusion [113]. This suggests that using ACh iontophoresis as a test of NO-dependent vasodilation is somewhat oversimplified. Moreover, additional methodological issues are related to the nonspecific, current-induced vasodilation. This is particularly true in patients with diabetes, in whom neuropathy may decrease the vasodilatory response to C-fibers activation (axon reflex). Yet, different iontophoresis protocols have yielded varied conclusions in patients with type 1 diabetes, highlighting the importance of considering these methodological issues analytically [67].

In recent years, the development of new noninvasive methods that assess the cutaneous microcirculation have provided tools that might have potential value to predict or evaluate the wound healing process [19]. Among them, hyperspectral imaging (HSI) allows for measurement of cutaneous oxygen saturation over a wide area (Fig. 10.4). In the lower limb of patients with diabetes and DFU, there was a negative association between tissue oxygenation assessed by HSI at baseline and healing at 12 weeks [114]. An index derived from HSI measurements indicated very good sensitivity and specificity for predicting healing of ulcerations in a small group of patients with type 1 diabetes [115], which needs to be validated on a larger scale.

The most recently developed laser-based imaging technology, laser speckle contrast imaging (LSCI), allows for near real time analysis of cutaneous blood perfusion [8]. Based on the same fundamental operating principles as LDF and LDI, the LSCI head unit emits a laser beam above the skin surface from which speckle pattern images are acquired

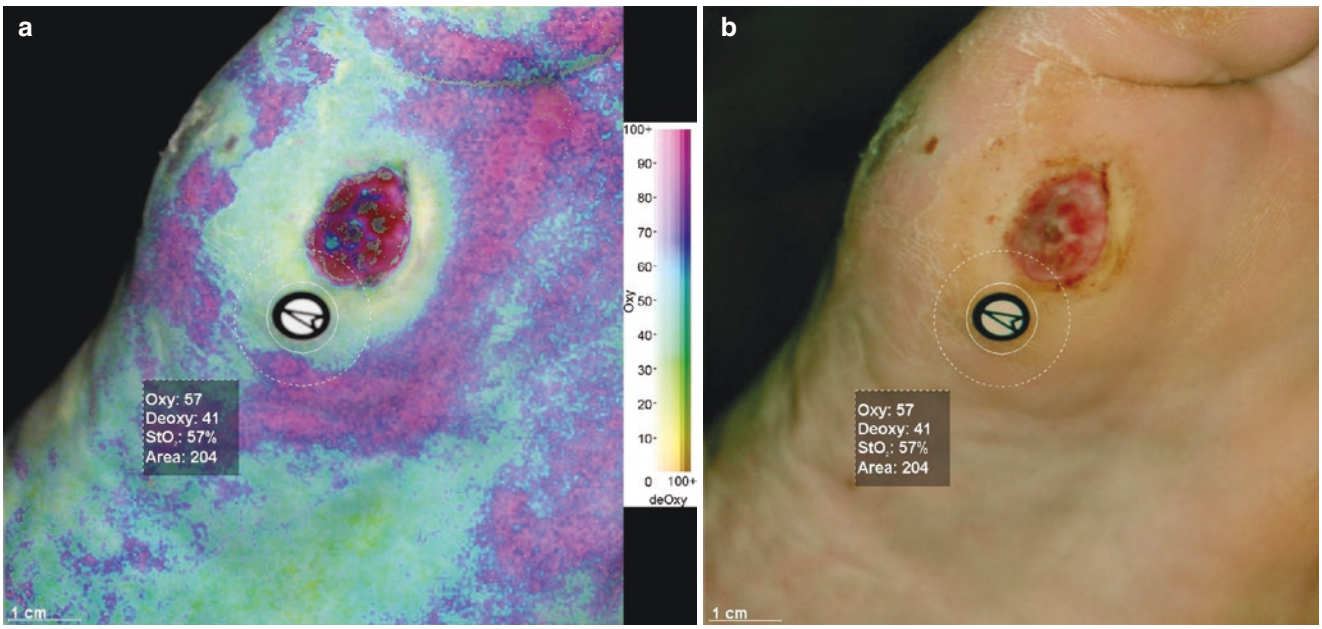

Fig. 10.4 Measurement of cutaneous oxygenation in a peri-wound area using hyperspectral imaging (**a**) and a corresponding photo of the ulcer (**b**). The image shows poor skin oxygenation around the wound

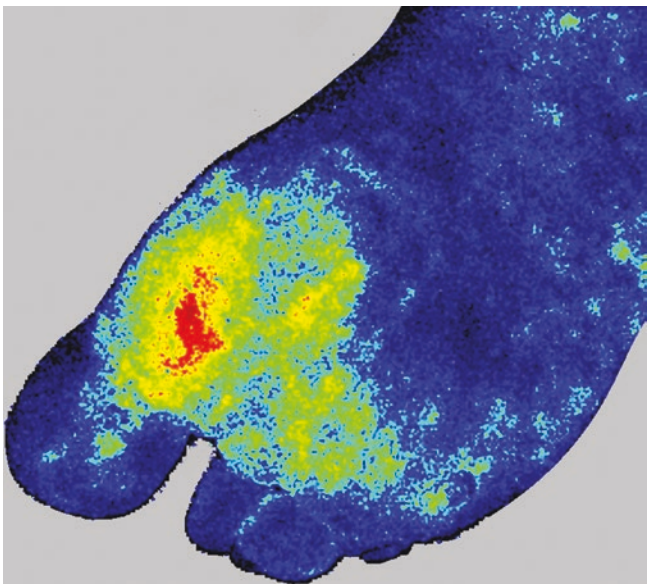

Fig. 10.5 Measurement of skin perfusion with laser speckle contrast imaging on the plantar region of the foot in a diabetic patient with an active ulcer that is healing properly. Colors range from dark blue (no perfusion) to red (high perfusion)

to provide a perfusion index proportional to the mean velocity of red blood cells [116]. Given that LSCI continuously measures cutaneous microvascular blood perfusion over a large area (> 100 cm^2) using a high sampling frequency, it theoretically combines the primary advantages of LDF and LDI, reducing the spatial variability and temporal variability associated with each technology, respectively (8116) (Fig. 10.5). Recent research has established the relevancy of LSCI for monitoring the health of the superficial microvasculature in an animal model of pressure ulcer [117], and for assessing the effect of chronic leg ulcer treatment in patients with sickle cell disease [118]. Further data regarding diabetic foot ulcers should be published in the next few years.

Conclusion

In conclusion, structural and functional impairment of the cutaneous microcirculation in diabetes contributes to complications such as foot ulcerations. Despite recent advances in the methods of assessing skin blood flow, the study of cutaneous microvascular function in humans remains challenging. Indeed, the pathological processes appear to evolve over the time course of the disease. Moreover, there is heterogeneity among the different vascular beds (e.g., glabrous vs. non-glabrous skin, lower vs. upper limb). Most studies so far have relied on laser Doppler coupled with iontophoresis of vasodilating substance, a technique that does not allow for discrimination between endothelial vs. neurovascular dysfunction. The complex relationship between sensory nerves and endothelium-dependent vasodilation involves recently discovered pathways that may play a role in diabetic microvascular dysfunction in the skin. Further research is needed to clarify their role and determine whether they could be used as potential therapeutic targets to prevent diabetic foot ulcers or improve their healing rate.

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Structural and Functional Changes in Skin of the Diabetic Foot

11

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Abstract

Diabetes, especially type 2, is characterized by systemic inflammation. At the skin level, there is increased infiltration by inflammatory cells and polarization of the macrophages toward the M1 inflammatory type. In addition, there is increased expression of MMP-9 and protein tyrosine phosphatase-1B (PTP1B). Other dermatologic conditions include acanthosis nigricans, characterized by a hyperpigmented, velvety, cutaneous thickening that appears predominantly in the neck, axilla, and groin areas; necrobiosis lipoidica (NL), a chronic, necrotizing, granulomatous skin disease; granuloma annulare; diabetic bullae; and diabetic dermopathy. As these conditions can be present in the lower extremity, they should be sought and easily recognized by the health care providers who manage the diabetic lower extremity.

Dermal Extracellular Matrix in DM

The dermis consists of a thin superficial portion, known as the papillary dermis, and a wider, deeper area known as the reticular dermis. The epidermis binds the papillary dermis superiorly, the epidermal ridges laterally, and inferiorly by the superficial vascular plexus and the reticular dermis, which lies between the papillary dermis and the subcutaneous fat.

The papillary and reticular dermis contains collagen, reticulin, and elastic fibers embedded in a ground substance, and are also called dermal matrix. The dermal matrix fills the spaces between the fibers and contains mainly glycoproteins, water, electrolytes, and plasma proteins (Fig. 11.1). Collagen provides the skin with tensile strength. Twenty-nine types of different collagen have been described in humans; however, more than 90% of the body's collagen is represented by types I, II, III, IV, and V. Type I accounts for approximately 80% of the total amount of dermal collagen and is found in the large fiber bundles of the reticular dermis. Depletion of type

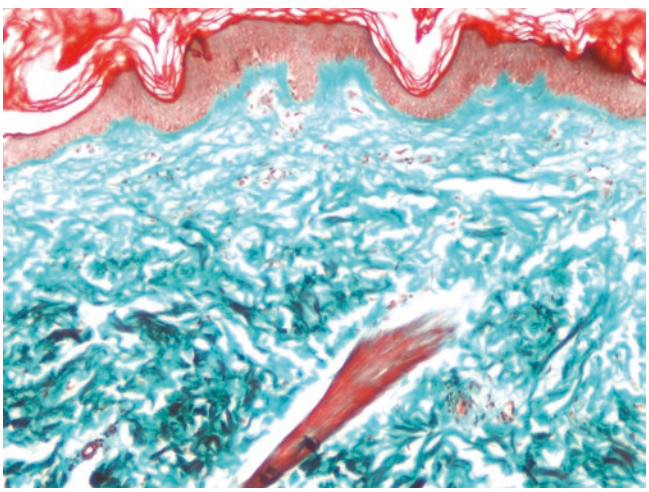

Fig. 11.1 Normal skin. Masson's trichrome stain shows thick collagen bundles in dermis ×100

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I procollagen in human leg skin has been reported in diabetic patients, both in the absence and in the presence of complications, with depletion being worse in patients with ulcers [1]. Additionally, unlike diabetic patients without complications, significant disarray of the dermal collagen bundles has been reported after light microscopic analysis of the skin of patients with foot ulcers [1] . A generally disorganized dermis has also been visualized by scanning electron microscopy of diabetic skin of 12-week-old Tsumura-Suzuki obese diabetic mice with smaller and less dense fibers [2]. These data, in addition to indicating pathological deposition, also indicate collagen reduction in diabetic skin. Similarly, decreased expression and production of dermal type I collagen has recently been described in Alloxan-treated mice with overt DM and also in mice with blood glucose fluctuations [3]. Type III, also known as fetal collagen or reticulum fibers, represents up to 10% of dermal collagen. This type of collagen is prevalent during fetal life; however, in post fetal life it is limited to the papillary and adventitial dermis. Furthermore, it serves as a framework on which type I collagen is synthesized. Decreased collagens I and III content in diabetic skin are associated with a reduced collagen I/III ratio. However, this decreased collagen content is associated with increased gene expression of enzymes involved in collagen synthesis and decreased production of factors that promote collagen degradation. This suggests that the defect in collagen protein content in diabetic skin at baseline is at the posttranscriptional level [4]. A lower I/III collagen ratio has been associated with reduced connective tissue stability [5], which might explain the lower mechanical stability of diabetic skin.

Another important component of the human dermal matrix, namely hyaluronic acid, has been studied in human skin in patients affected by insulin-dependent DM. A considerable reduction in hyaluronic acid, particularly in the region of the dermal epidermal junction, has been found in the dermis of patients with low joint mobility, whereas in patients with little or no impairment of joint mobility, hyaluronic acid distribution predominantly resembles that of the normal condition [6].

Skin Inflammation in DM

The normal mammalian response to skin injury occurs in three overlapping but distinct stages: inflammation, new tissue formation, and remodeling. Inflammation, the first stage of wound repair, occurs immediately after tissue damage, and components of the coagulation cascade, inflammatory pathways, and immune system are needed to prevent ongoing blood and fluid losses, to remove dead and devitalized tissues and to prevent infection. Dysregulated inflammation is one of the primary pathologies associated with chronic wounds, thus an understanding of the causes and consequences of dysregulated inflammation in diabetes is key if effective therapies are to be developed. Recent studies of the role of inflammation in diabetic wounds mainly focus on two kinds of skin inflammatory cells (macrophages and mast cells) in the dermis, and systemic or local inflammatory cytokines in patients serum and skin.

Macrophages

During the inflammatory phase of wound healing, macrophages are recruited to the wound site, involved in host defense, the initiation and resolution of inflammation, growth factor production, phagocytosis, cellular proliferation, and tissue restoration in wounds. They display impressive plasticity, as they express a polarization of classic (M1) and alternative activation (M2) phenotypes, which are mediated by cytokines, oxidants, lipids, and growth factors [7]. During normal wound healing, macrophages demonstrate transitions in phenotype and function in tissue repair progression, although the factors that regulate these transitions remain poorly defined. Thus, M1-activated macrophages initiate an acute inflammatory response, whereas during the proliferative phase M2 macrophages promote angiogenesis and granulation tissue formation [8]. In contrast, wounds in diabetic mice are characterized by a paradoxical and damaging delay in essential macrophage response in the early phase and by the prolonged accumulation of macrophages associated with increased levels of pro-inflammatory cytokines and proteases and reduced levels of various growth factors in the later phase [9, 10]. These conditions contribute to impaired diabetic wound healing. Studies in our unit have shown that diabetic rabbit skin has a higher number of M1 and lower number of M2 macrophages at baseline, before the creation of a wound. Although there was no difference in the number of M1 macrophages between nondiabetic and diabetic (ischemic and neuroischemic) wounds, there were lower number of M2 macrophages in all of the diabetic wounds leading to an overall higher M1/M2 ratio in the diabetic wounds suggesting a chronic wound environment [11]. Similar results were also found in the diabetic mouse model in our unit, with higher M1/M2 ratio in not-injured skin [12]. Similar results were also noticed in human diabetes. More specifically, we found that in the forearm not-injured skin, the number of M1 macrophages was increased in subjects with diabetes, whereas the number of M2 macrophages and the M1/M2 ratio were not different. In the foot skin, the number of M1 macrophages tended to be increased in subjects with diabetes, whereas the number of M2 macrophages tended to be reduced, resulting in a higher M1/M2 ratio. Moreover, mRNA expression of the M1-associated pro-inflammatory cytokines TNF-α and IL-1β was elevated in diabetic foot skin, whereas the M2-associated anti-inflammatory cytokine IL-10 is reduced [13] (Fig. 11.2).

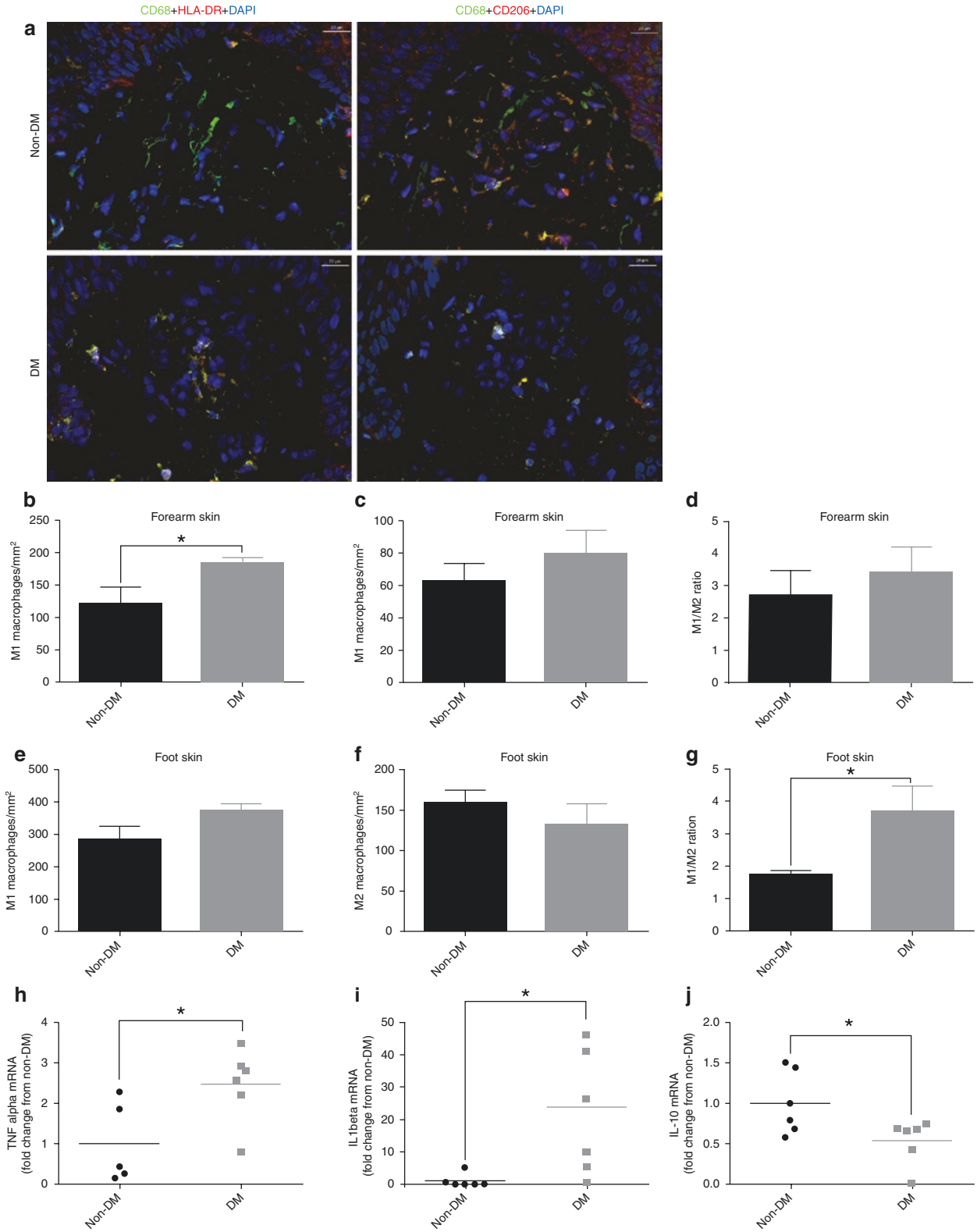

Fig. 11.2 Skin macrophage phenotype in DM patients [13]. (**a**) Representative images of M1 and M2 macrophages in foot skin biopsies from non-DM and DM subjects (scale bar: 20 μm). M1 (left panel) and M2 (right panel) are shown in yellow-orange as a result from the triple positive staining. (**b–d**) In the forearm skin, (**b**) more macrophages existed in DM whereas (**c**) the number of M2 macrophages and (**d**) the M1/M2 ratio was not different. (**e–g**) In the foot skin, (**e**) the number of M1 macrophages tended to be increased in DM, whereas (**f**) the number of M2 macrophages tended to be reduced, resulting in (**g**) a higher M1/M2 ratio. (**h–j**) mRNA expression of the M1 markers (**h**) TNF-α (I) and IL-1β was increased, whereas the M2 marker (**j**) IL-10 was reduced in foot skin of DM patients. *$p < 0.05$

Macrophage wound therapy, via in situ activation, recruitment, or application of exogenous macrophages to wounds, has been explored for its use in chronic wounds through stimulation of proliferation and angiogenesis, influence on protease imbalances, and increased phagocytosis [14] . A current trend in macrophage therapy research shifts the focus from inhibiting macrophages to the wound site to emphasizing controlled recruitment and modulation. Jumpstarting macrophage infiltration into diabetic wound in the early phase and polarizing the macrophages from the M1 phenotype to the M2 phenotype can be both effective means to promote wound healing in diabetic mice [10, 15]. M1 macrophages have been shown to secrete IL-1β in diabetic wounds of both humans and mice, which blocks the activation of the M2 phenotype. Inhibition of IL-1β in vivo by a neutralizing antibody leads to downregulation of pro-inflammatory cytokines, the upregulation of healing-associated growth factors and the switching of macrophages to the M2 phenotype with concurrent resumption of the healing process [15]. In our unit, we have shown that Substance P treatment of wounds in diabetic mice induced an acute inflammatory response with increased M1 macrophage activation in the early stages which was followed by the progression to the proliferative phase and modulated macrophage activation toward the M2 phenotype that promoted wound healing [12]. In addition, we have also reported that treatment of diabetic mice with the mast cell stabilizer disodium cromoglycate (DSCG) improves the diabetes-associated impaired wound healing and shifts macrophages to the regenerative M2 phenotype [13] .

Inflammatory Cytokines

Diabetes, especially type 2, and obesity are associated with increased systemic inflammation, as described by the elevated circulating inflammatory cytokines [16]. Inflammation in the adipose tissue has been proposed as one of the main factors that lead to the development of insulin resistance and type 2 diabetes [17]. However, there is limited information regarding connection between systemic inflammation and at the skin level and the development of diabetic foot ulceration. In order to examine the role of vascular function and inflammation in the development and failure to heal diabetic foot ulcers (DFUs), a study in our unit followed a cohort of diabetic patients for an average period of 18 months [18]. DFUs developed in 29% diabetic patients. All patients who developed ulceration had more severe neuropathy, higher creatinine and white blood cell count, and lower endothelium-dependent and -independent vasodilation in the macrocirculation than those who did not. Complete ulcer healing was achieved in 53% DFUs patients during the first 12 weeks, whereas remaining 47% patients did not heal. There were no differences in the above parameters between the patients who healed their DFU and those who did not, but patients whose ulcers failed to heal had higher tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), matrix metallopeptidase 9 (MMP-9), and fibroblast growth factor 2 (FGF-2) serum levels when compared with those who healed.

Skin biopsy analysis from forearm skin and foot skin showed that compared with control subjects, diabetic patients had increased immune cell infiltration, expression of MMP-9, and protein tyrosine phosphatase-1B (PTP1B). MMP-9 is mainly released by inflammatory cells and is involved in the breaking down of matrix proteins and growth factors [19]. At skin level, MMP-9 expression by inflammatory stromal cells was also higher in the diabetic patients, suggesting that these cells may be a source for the observed increased systemic levels in the same population. PTP1B is a ubiquitously expressed protein tyrosine phosphatase that localizes in the endoplasmic reticulum, is upregulated by inflammation, and negatively regulates the signaling of insulin, leptin, and various growth factors that are involved in wound healing, such as VEGF, EGF, PDGF, and TGF-β [20]. Increased PTP1B expression was present in all prominent skin cell populations of diabetic patients, whereas patients whose ulcers failed to heal had marginally increased expression in the endothelial cells when compared with patients whose ulcers healed. Taken in context, these results indicate that there is increased extracellular MMP-9 and intracellular PTP1B expression, leading to local inactivation and resistance to the action of various growth factors that are involved in wound healing. Furthermore, this leads to increased levels of circulating growth factors in a way that is similar to the increased insulin levels in situations of insulin resistance.

In another study we also studies patients with an active DFU [21]. DFU patients had higher levels of growth-related oncogene (GRO), interleukin-8 (IL-8), macrophage-derived chemokine (MDC), tumor necrosis factor alpha (TNF-α), c-reactive protein (CRP), SDF-1, and stem cell factor (SCF). In addition, patients who healed their ulcers had lower serum CRP and Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) at the beginning of the study 1 and lower interleukin-1 alpha (IL-1α) at the beginning and the end of the study. Serum SDF-1 levels were increased in DFU patients while its expression in forearm skin biopsies was increased in diabetic patients with or without DFU. No major changes were observed in the expression of its receptor CXCR4 at both forearm and foot skin specimens. These results indicate that CRP, IL-1α, and GM-CSF can be useful as prognostic markers for DFU healing.

Dermatologic Conditions in DM

Acanthosis Nigricans

Acanthosis nigricans (AN) is one of the most recognized skin manifestation of diabetes. It is observed in fully 74% of obese patients and becomes a reliable cutaneous marker

of hyperinsulinemia in obese individuals [22]. AN is a symmetric eruption characterized by a hyperpigmented, velvety, cutaneous thickening that appears predominantly in the neck, axilla, and groin areas. The histological findings are papillomatosis and hyperkeratosis, characterized by irregularly folded epidermis, exhibiting various degrees of acanthosis. Typically, the dermal papillae are projected upward, and the valleys in between them show mild to moderate acanthosis and filled with keratotic material. The epidermis at the top and at the sides of the papillae appears thinned and the brown color of the lesions is due to the thickening of keratinin-containing superficial epithelium. There are seven types of acanthosis nigricans: hereditary benign AN, obesity-associated, syndromic, malignant AN associated in particular with abdominal adenocarcinoma (gastric carcinoma), acral or benign AN, drug-induced (nicotinic acid and corticosteroids), and mixed. AN is a chronic but reversible condition. In obesity-associated AN the pathogenesis is related to high levels of circulating insulin, bound with insulin-like growth factor receptors and stimulates keratinocyte and dermal fibroblast growth. In the malignant form of AN the associated growth factors secreted by underlying malignancy are believed to result in cutaneous changes of AN. Treatment consists of treating the underlying cause. In the diabetic patient, weight control, dietary restrictions, and increased physical activity are of primary importance and have been proved to be most effective in controlling AN [23]. Topical keratolytics (e.g., salicylic acid, retinoic acid, and ammonium lactate) and oral isotretinoin can reduce thicker plaques in areas of maceration, decreasing odor and discomfort [24]. Other treatment options include both laser therapy and surgical excision.

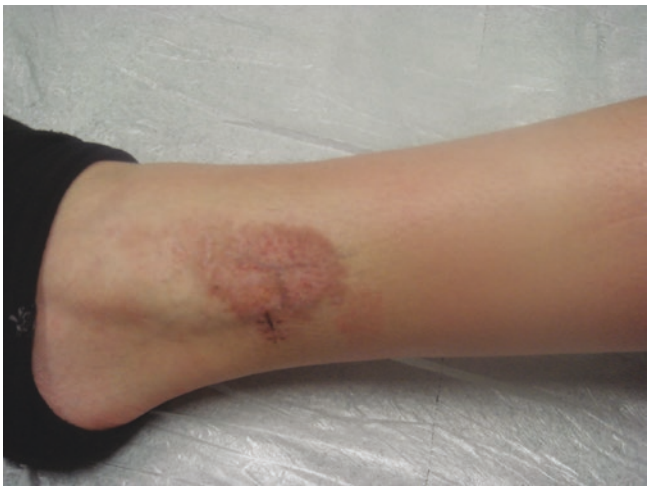

Fig. 11.3 NLD in a 16-year-old girl with T1DM and a patch on the leg with an atrophic, depressed, slightly yellow center, and well-defined raised purple edge

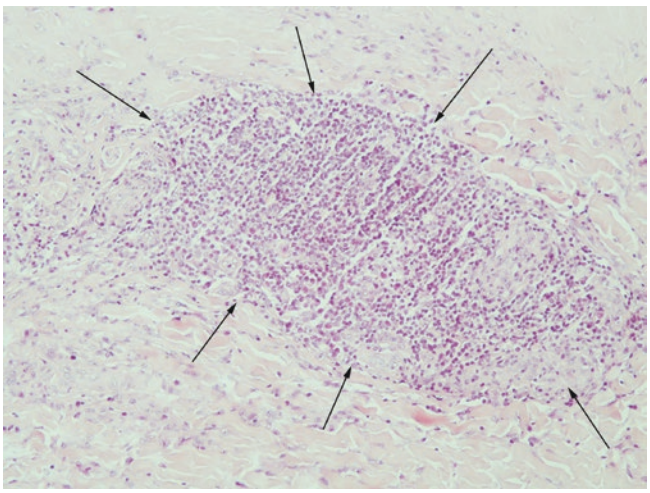

Fig. 11.4 Patchy lymphoplasmacytic infiltration around blood vessels (arrows point to plasma cells). (H&E ×200)

Necrobiosis Lipoidica

Necrobiosis lipoidica (NL) is a chronic, necrotizing, granulomatous skin disease that occurs primarily in individuals with diabetes. It appears in the form of red non-scaling patches or plaques sharply demarcated with irregular contours. The edges are elevated, erythematous, and slightly indurated; the center of the lesion is atrophic, yellow-brownish, and may ulcerate (Fig. 11.3). Lesions often start out small, but have a tendency to grow to several centimeters in diameter. The ulceration is relatively frequent if lesions are large but perforation is generally rare. They may be single or multiple, most commonly distributed bilaterally on the lower extremities, particularly the pretibial areas, but may occur on the face, trunk, and upper extremities as well. Histologically the whole of the dermis is affected by palisaded granulomatous inflammation sparing the epidermis. The inflammation often spreads into subcutaneous septae giving the false impression of subcutaneous paniculitis. Collagen degeneration without mucin component is demonstrated in the central of the lesion. Furthermore, the periphery of the main lesion usually exhibits sclerosis and sometimes lipid droplets associated with foam histiocytes are evident. In deep dermis, lymphoid follicles and plasma cells may be present. The latter are consider to be a strong histological finding confirming the histological diagnosis. The differential diagnosis includes palisaded granulomatous dermatitis, among them being granuloma annulare, rheumatoid nodule, and necrobiotic xanthogranuloma (NX). In NL the degenerated collagen is pale, acellular, and horizontal in its distribution. This pattern has been linked to the appearance of a layer cake (Fig. 11.4). Rheumatoid nodule granulomas tend to be larger and are usually located over bony prominence, near joints. Histologically they are located in the deep dermis, or in subcutis enclosing a central

area with fibrin, which is homogeneously eosinophilic lucking mucin. NX typically shows a periorbital predilection. Histological findings for NX include an inflammatory mixed cellular population with Touton type giant cells, foamy histiocytes, and necrotic areas with neutrophilic debris involving the dermis and subcutaneous tissue [25] . The cause of NL is unknown, but there are several proposed theories behind the pathophysiology of NL such as microangiopathic changes, abnormal collagen, altered lipid metabolism, and impaired immunity. The combination of microangiopathy, neuropathy, and the release of inflammatory cytokines leads to the destruction of the collagen matrix, resulting in sclerosis and granulomas formulation. Excess lipid deposition in the dermis has as a result the yellow appearance [26]. The mainstay of treatment is currently steroids, either topical, intralesional, or rarely systemic. Steroids are cost-effective and have low side-effect profiles. Other treatments include systemic cyclosporin or ticlopidine [27], CO_2 laser therapy [28], and platelet-rich plasma [29]. Lesions may be excised with skin grafting, but patients with diabetes may be poor surgical candidates. Most recently, TNF-α antagonists, such as enteracept and infliximab, have been selected as possible therapies [30, 31].

Generalized Granuloma Annulare

Granuloma annulare (GA) is an idiopathic, benign, and asymptomatic granulomatous condition that is more frequently seen in women rather than men. The exact etiology and pathogenesis of GA remains unknown. Several mechanisms have been implicated in the pathogenesis of GA such as cell-mediated immunity, vasculitis, abnormalities in macrophage function, and primary collagen degeneration. Possible trigger factors include infections, sun light exposure, and hepatitis B vaccine. Clinical subtypes include localized, subcutaneous, perforating, generalized, and papular GA. Only the generalized form shows consistent and significant correlation with diabetes across most studies and 21–77% of patients with generalized GA have diabetes (predominantly type 2 diabetes) [32] . Generalized GA is an inflammatory lesion that usually takes the form of multiple, small, firm, skin-colored or red dermal papules in an annular arrangement that tend to be found on the distal extremities (Fig. 11.5). The skin lesions of GA are similar to necrobiosis lipoidica but GA does not exhibit epidermal atrophy and yellow discoloration. The histopathology shows lymphohistiocytic granulomatous inflammation of the dermis and collagen degeneration (Fig. 11.6). Colloidal iron stain reveals abundant deposition of mucin. The presence of mucin and the absence of plasma cells help to distinguish histologically GA from NL. Treatment is similar to NL.

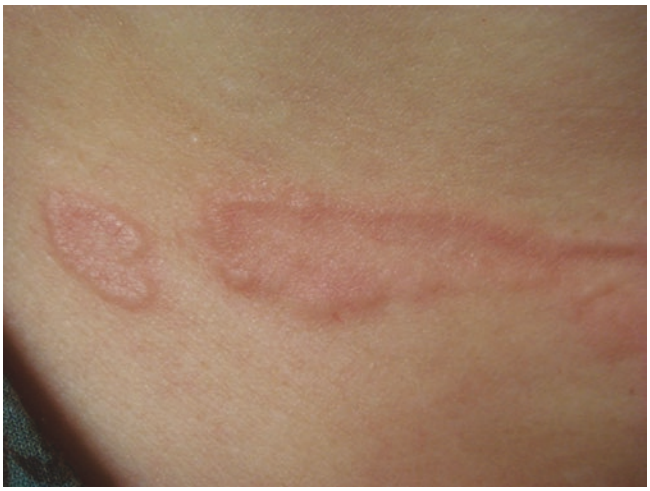

Fig. 11.5 Granuloma anulare. Skin punch biopsy from the abdomen of a 40-year-old woman with T2DM (erythematous annular plaques)

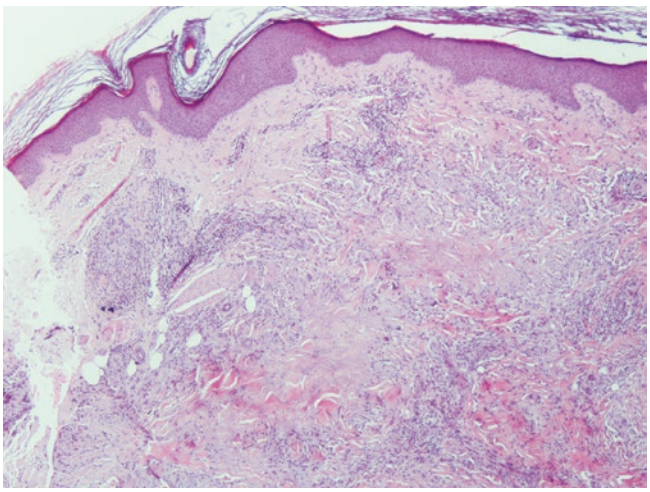

Fig. 11.6 Granuloma anulare. H&E (×100) shows necrobiotic granuloma surrounded by lymphocytes and histiocytes

Diabetic Bullae

Diabetic bullae (DB), or bullosa diabeticorum, is a rare, noninflammatory, bullous disorder characterized by tense, painless, bullae that develop abruptly on normal-appearing skin, primarily on the dorsa and the sides of the lower legs and feet, and less often affecting the hand or forearm. The blisters are noninflammatory in nature and heal in several weeks without scarring. The etiology of blister formation is unclear, but is postulated to involve trauma, ultraviolet (UV) light exposure, hypoglycemia or highly fluctuating blood glucose levels, an autoimmune condition, vascular insufficiency, neuropathy, and changes in calcium or magnesium metabolism [33, 34]. The microscopic findings are nonspecific and the level of the bullae formation is variable. The bullae contain fibrin and occasional inflammatory cells but

in case of subepidermal bullae the cavity is filled with blood. Immunofluorescence is not reliable in establishing accurate diagnosis and only helps to exclude other bullae diseases. The differential diagnoses include bullous pemphigoid, porphyria, and pseudo-porphyria, which can be ruled out by submitting a biopsy of the lesion for direct and indirect immunofluorescence [25] . Treatment of DB is focused on skin protection and preventing secondary infection. These lesions usually resolve without intervention within a matter of weeks. Uncomplicated blisters should be left intact, but sterile aspiration of fluid may prevent rupture in some cases. Ulcerated blisters should be treated with aggressive wound management [35] .

Diabetic Dermopathy

Diabetic dermopathy (DD), also known as spotted leg syndrome, is the most common cutaneous finding in diabetic and seen in about 40% of diabetic patients [24, 36]. It is characterized by atrophic, hyperpigmented, and irregularly shaped papules or plaques predominantly on the pretibial skin. The lesions are asymptomatic and may persist indefinitely or disappear without treatment. Diabetic dermopathy is a clinical diagnosis. The histopathology of the lesion is relatively nonspecific, while the fully developed lesions show epidermal atrophy with a mild perivascular lymphohistiocytic infiltrate and hemorrhage in papillary dermis. The pathogenesis of DD is unknown. Trauma and microangiopathy with associated capillary changes have been reported as contributing factors; however, the literature abounds with evidence that supports and refutes these potential factors [36, 37]. Currently, there is no recommended medical intervention for DD because the lesions are asymptomatic and may resolve spontaneously. Treatment should take into consideration the possibility of a secondary infection. Patients with these findings may also prone to other microangiopathic complications and coronary artery disease, because of their association with diabetic dermopathy [38].

Acquired Perforating Dermatosis

Acquired perforating dermatosis (APD) defines a group of chronic skin disorders characterized by transepidermal perforation and elimination of a dermal component of the skin [39] . This disorder is historically classified by the predominant dermal material identified microscopically such as keratin, collagen, or elastic tissue. Hence, APD can be divided into four types: (1) elastosis perforans serpiginosa, (2) reactive perforating collagenosis (Fig. 11.7), (3) Kyrle's disease, and (4) perforating folliculitis. APD is associated with diabetes, chronic renal failure, and dialysis, or a combination

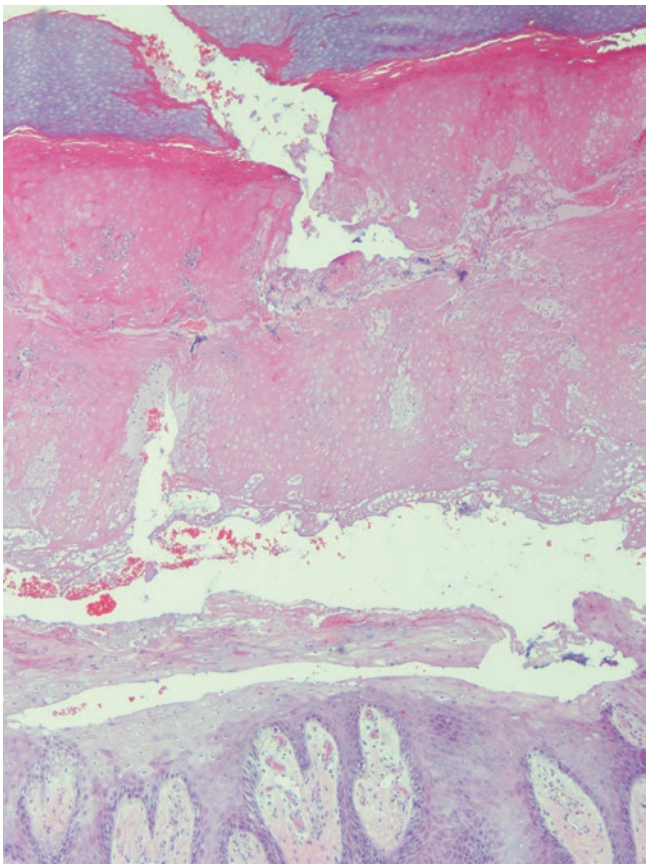

Fig. 11.7 Perforating collagenosis. Skin surgical biopsy. H&E (×100) shows skin biopsy from the foot. Altered collagen is eliminated through epidermis into the thick keratin layer

of these factors [40–42]. These lesions appear as pruritic, hyperkeratotic, dome-shaped papules and nodules, often with central umbilication, mainly occurring on the extensor surface of the limbs, but also on the trunk, hands, and face. Histologically, transepidermal channels traverse an acanthotic epidermis and are filled with keratin, pyknotic nuclear debris, inflammatory cells, elastin, and collagen depending on the nature of the underlying disease. They all share a common microscopic finding, which is the intraepidermal elimination of these substances. In elastosis perforans serpiginosa altered elastic fibers pass through channels from dermis to epidermis. The pathogenesis is not well understood, it mainly includes minor skin trauma from scratching, manifestation of microangiopathy, metabolic disorders causing an epidermal or dermal alteration, and a deposition of some substances not removed by dialysis, which the immune system then treats as foreign [43] . APD in the setting of diabetes is relatively unresponsive to therapy, but may resolve slowly if trauma and scratching are avoided. Therefore, the key treatment strategy is the symptomatic relief of pruritus. Further treatments with beneficial effects are reported, among these are topical keratolytics, topical and systemic

retinoids, allopurinol, PUVA, UVB phototherapy, topical and intralesional injection of steroids, antibiotics (doxycycline), oral antihistamine, cryotherapy, and renal transplantation [39, 44].

Diabetic Thick Skin

Diabetic patients often exhibit thickening of the skin caused by excessive accumulation of abnormal collagen. Clinically, thickened skin is divided in three distinct categories: (1) asymptomatic benign skin thickening; (2) scleroderma-like skin changes in the fingers with limited joint mobility, also known as diabetic hand syndrome or limited joint mobility syndrome; and (3) diabetic scleredema. Thickened skin is thought to be a manifestation of the abnormal glycosylation of collagen occurring in a hyperglycemic state, leading to increased cross-linking of collagen fibers that become resistant to degradation by collagenase [45]. Others have suggested that excess insulin acting as a growth factor promotes collagen proliferation [46].

A scleroderma-like syndrome develops in 8–50% of diabetic patients. Skin on the dorsal part of the hand can thicken, resulting first in stiffness of the metacarpophalangeal and proximal interphalangeal joints of the fingers [45]. Thickened skin on the fingers, known as Huntley's papules or finger pebbles, appears as grouped indurate papules on the extensor surface of the fingers, knuckles, or periungual area. Decreased joint mobility is manifested as a limited ability for active extension, but later in the course of this syndrome, limited flexion may occur. The literature suggests that diabetic hand syndrome is a cutaneous marker for the development of diabetes-related microvascular complications [37, 45] .

Diabetic scleredema, is a rare chronic connective tissue disorder primarily associated with type 2 diabetes. It consists of a dramatic increase in the thickness of the reticular dermis, initially on the face, and extends to the posterior neck and upper back. Diabetic scleredema is usually asymptomatic, but neck discomfort and back pain may occur, especially in more severe cases [47] . The affected skin is hard, thick, and indurated, sometimes erythematous, and may have a peau d'orange appearance. Unequivocal diagnosis of scleredema by histologic examination requires a full thickness excisional biopsy to examine the dermis. Histologically, diabetic scleredema reveals a markedly thickened reticular dermis, increased mast cells, thick collagen bundles and accumulated hyaluronic acid between the collagen bundles, and there is no sign of edema nor sclerosis [48]. The diagnosis is usually based on history and physical examination. Histologically special stains such as Alcian blue and Colloidal iron reveal interstitial mucin deposits in between the collagen bundles. There is no highly effective treatment for diabetic scleredema. Therapies include potent intralesional glucocorticoids, low-dose methotrexate, and UV light phototherapy. Although strict glycemic control does not show consistent therapeutic benefit in scleredema diabeticorum, it is proposed to be an effective preventive measure [49–51].

Eruptive Xanthomas

Eruptive xanthomatoses are rare and occurs more often in patients with poorly controlled type 2 diabetes, characterized by a collection of lipids in the skin. They arise suddenly in groups of multiple yellow papules with surrounding erythema, commonly located on the extensor surfaces of the extremities and on the buttocks. The histopathology shows an accumulation of lipid-laden histiocytic foam cells with a mixed infiltrate of lymphocytes and neutrophils in the dermis. These lesions can be the first sign of diabetes. The reason for the increased frequency of eruptive xanthomatosis among individuals with diabetes has been well characterized. Insulin is a stimulating factor critical to the normal activity of lipoprotein lipase, the insulin-deficient state of insulin-dependent diabetes results in the decrease in lipoprotein lipase activity and then leads to an accumulation of serum triglycerides. Occasionally, when the serum triglyceride level reaches 2000 mg/dL, lipids will deposit in the skin [52]. Eruptive xanthomas are a cutaneous manifestation of the hyperlipidemic state, especially hypertriglyceridemia. Early identification of xanthomatoses can facilitate timely treatment and possible avoidance of more serious manifestations of hyperlipidemia such as atherosclerotic complications and pancreatitis. Systemic drugs that lower lipid levels aid in both resolving the lesions and preventing other complications of hyperlipidemia, such as coronary artery disease and pancreatitis [33, 47].

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Biomechanics of the Diabetic Foot: The Road to Foot Ulceration

12

Panagiotis V. Tsaklis and Nikolaos Tentolouris

Abstract

Biomechanics is a branch of the life sciences for the study of the structure and function of biological systems including humans by means of the methods of mechanics. Biomechanics is clearly relevant to diabetic foot, since the majority of the feet injuries are related to the mechanical stress applied to the structures of the feet. Thus, callus is formed in the feet when increased pressure is applied for a prolonged period of time; an ulcer will not heal if there is no sufficient offloading; and callus or ulcers will recur if there is no proper offloading of the vulnerable areas of the feet. Knowing, therefore, the basic biomechanics of the foot is important for understanding the mechanism of development of ulcers, for organizing prevention methods, for the treatment of ulcers, and for prevention of relapses. For the Biomechanical and functional evaluation of the diabetic foot and the general mobility of the person, must be a holistic approach which include: the morphological investigation of the foot; the mobility measurements (Range of Motion) of the foot joints; the recording and evaluation of the pressures around the plantar area and the weight distribution between legs (weight shift %); the Gait assessment and evaluation through kinematic and kinetic analysis of the movement of the foot and other body segments, like pelvis and trunk; and the assessment of the static and dynamic balance.

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Foot Anatomy and Function Related to Biomechanics

The human foot is a complex and strong mechanical structure containing 26 bones, 33 joints, and more than a 100 muscles, tendons, and ligaments. The feet support the weight of the body, and provide support during standing and fulcrum during walking. One of the principal functions of the foot is its shock-absorbing capability during walking or running. Furthermore, the foot has the particularity of forming arches which help to fit even on uneven surfaces [1].

The ankle joint is the major point for controlling sagittal plane movements of the leg relative to the foot, which is essential for bipedal ambulation [1]. The subtalar joint allows movement three planes described as pronation (combination of inversion abduction, and dorsiflexion) and supination (a combination of inversion, adduction, and plantar flexion) [2, 3]. The midtarsal joint, represents the functional articulation between the rearfoot and midfoot. The interrelationship of the subtalar and midtarsal joint provides full pronation and supination motions throughout the foot. The first metatarsophalangeal joint (MTPJ) incorporates the first metatarsal head (MTH), the base of the proximal phalanx, and the superior surfaces of the medial and lateral sesamoid bones within a single joint capsule. The main motion of the first MTPJ and the lesser MTPJs is in the sagittal plane (dorsiflexion and plantar flexion) (Fig. 12.1).

During propulsion the body weight is moving forward over the hallux creating dorsiflexion of the first MTPJ. This occurs with the hallux planted firmly on the ground and with the heel lifting for propulsion. The force acting across the first MTPJ approximates body weight, whereas the force across other MTPJs is considerably less [4]. Maximum load-

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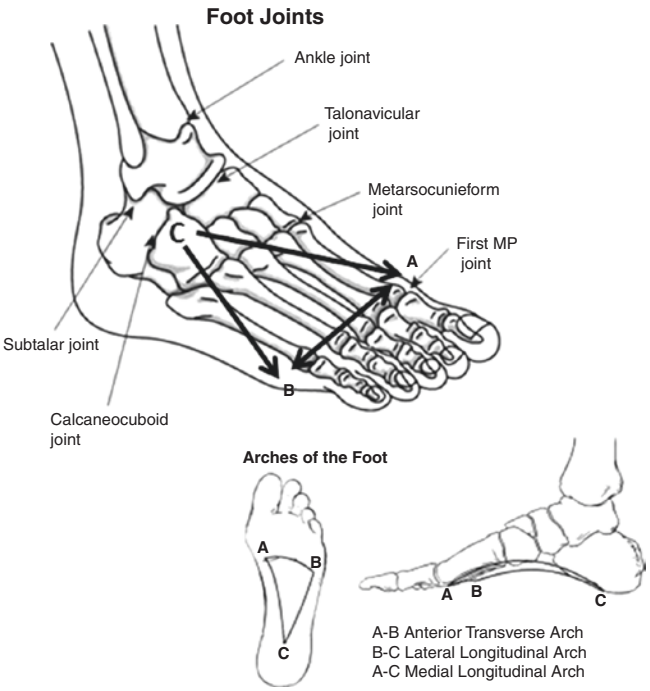


Fig. 12.1 Foot anatomy—joints and arches

ing of the first MTH and hallux is practically at the same time during stance in normal gait, highlighting the importance of the load-bearing function of both the hallux and first MTH.

The gait or walking cycle is a repetitive pattern involving steps and strides. A step is one single step, a stride is a whole gait cycle. The step time is the time from one foot hitting the floor to the other foot hitting the floor. Step width can be described as the mediolateral space between the two feet (Fig. 12.2b). The gait refers to a series of events which the leg experiences during ambulation. For analyzing gait cycle one foot is taken as reference and the movements of the reference foot are studied. Each leg experiences its own gait pattern which consists of two main phases: the stance phase (68% of the cycle) and the swing phase (38% of the cycle) (Fig. 12.2a).

During gait, the foot is required to be unstable for shock absorption and to adapt to the terrain, whereas during the propulsive phase the foot has to be stable to function as a lever. Foot flexibility and rigidity are mainly controlled with pronation and supination of the subtalar and midtarsal joints. As subtalar joint pronation after heel strike is a major shock-absorbing mechanism, limited joint mobility (LJM) or structural abnormality could compromise flexibility and shock absorption, thereby placing increased stress on the plantar skin surface [5, 6]. For example, LJM of the 1st MTPJ is the commonest cause of recurrent ulcers under the big toe. In addition, limited ankle dorsiflexion could result in increased pressure on the forefoot, in particular during the late stance phase of gait, caused by an early heel rise or compensatory pronation [5, 7, 8].

Beyond the pressure applied to the foot, another important mechanical quantity that contributes to the development of foot ulcers is the plantar shear stress, which results from the forces exerted parallel to the skin and tends to cause a tear [9, 10]. Ground reaction forces act in all three dimensions under the foot during locomotion. Among the three-dimensional stresses that act on the plantar surface, vertical stress (pressure) can easily be quantified via commercial pressure measurement systems [11]. Due to technical challenges, objective determination of the horizontal shear stress was not possible in the past, but during the last few decades, a variety of methods have been developed for its measurement [12]. Data suggest that peak plantar pressure and shear stress may occur at different anatomical sites of the diabetic foot and may explain why some ulcers do not develop exclusively at high-pressure locations [13, 14] (Fig. 12.8).

[A individual must be able to: support or assume upright position; maintain balance in an upright position during this dynamic situation; initiate gait from a static (zero-speed position) and control ambulation; develop new step forward; generate muscular forces; anticipate the gravitational forces; control the forces of momentum; sustain and distribute the Ground reaction forces (GRFs)]

Changes in the Foot Caused by Diabetes

Diabetes mellitus affects the structure and function of the foot. Many abnormalities which are a consequence of diabetes including chronic sensorimotor neuropathy, foot deformities, callus formation, limited joint mobility (LJM), high plantar pressures, and autonomic dysfunction interact and contribute to the development of foot ulceration. In addition, diabetes affects the skin, tendons, muscles, and periarticular tissues.

Foot Deformities

Foot morphology, even without a specific pathology, determines the biomechanical behavior and functionality of the foot. It is very important to consider both foot biomechanics and morphology when planning a foot offloading with orthotics and/or special shoes, in order to reduce plantar ulcer formation and thus avoid amputation in people with diabetes [15].

Sensory neuropathy with loss of protective sensation allows abnormal mechanical forces to cause painless injury to the skin or asymptomatic bone fracture [13, 16]. Motor nerve involvement causes muscle weakness and atrophy with foot imbalance leading to clawing of the toes, a high arched foot,

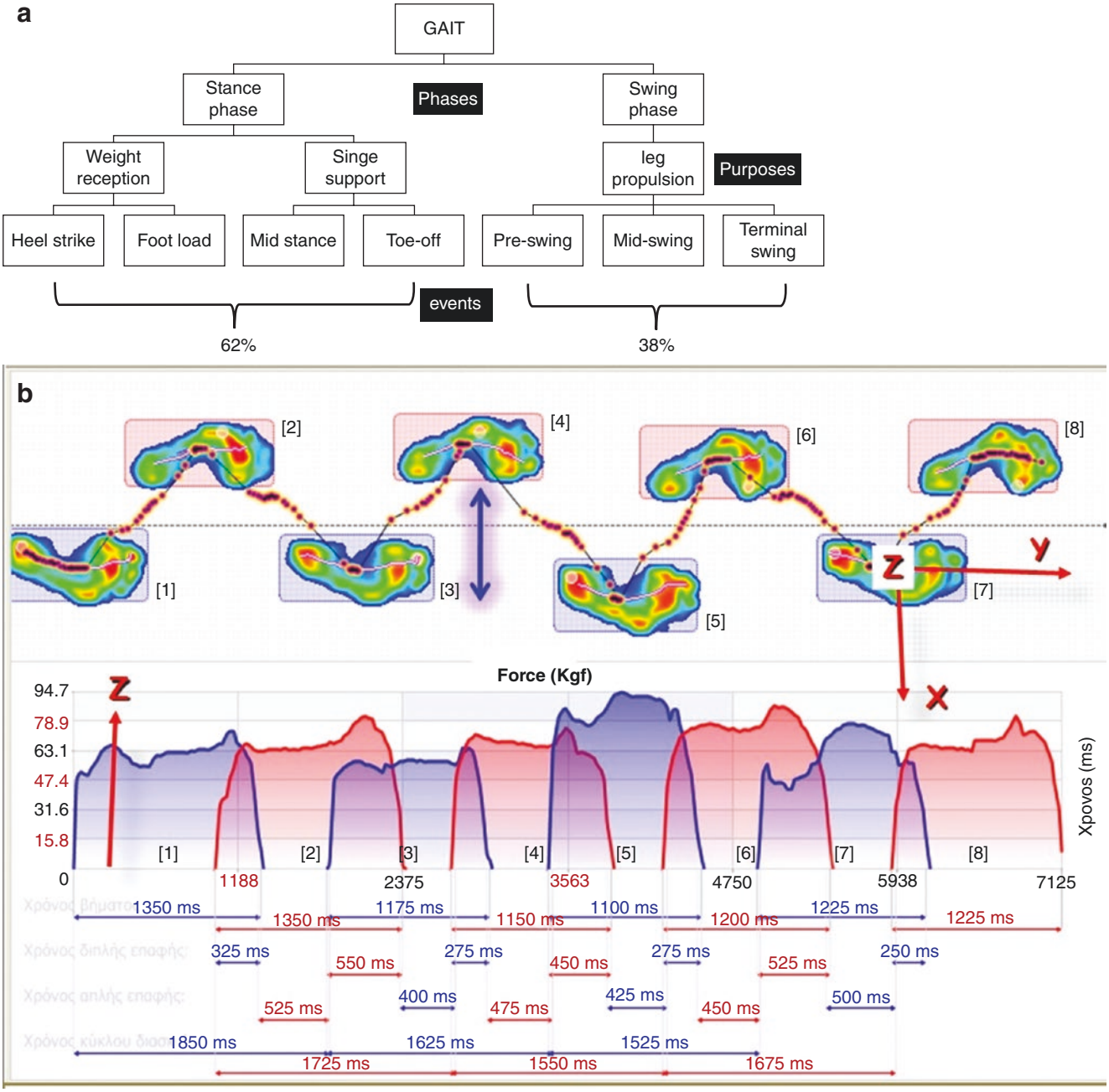


Fig. 12.2 (**a**) The GAIT cycle and events. (**b**) Spatiotemporal data during GAIT

or high pressure of the plantar surface [17–19]. Muscular atrophy has been defined as histological replacement of normal muscle by fat cells as a consequence of denervation [20]. Suzuki et al. found that neurogenic atrophy was associated with fatty infiltration of plantar muscles and impaired energy metabolism in ulcerated [21]. In addition, foot deformities are associated with and predictive of increased plantar pressures and foot ulceration [22, 23]. Prominent MTHs have traditionally been attributed to weakness of the intrinsic muscles of the foot leading to toe deformities [19]. Moreover, fat cushions under MTHs which are imbedded in the flexor tendons are believed to migrate distally with clawing of the toes, leaving the MTHs relatively unprotected [24]. Diabetic neuropathic patients with a toe deformity have a greater reduced sub-MTH padding compared with people without this deformity, indicating increased probability of high pressure and risk for foot ulcer development at these sites [25]. Sensory neuropathy is often emphasized in considerations of

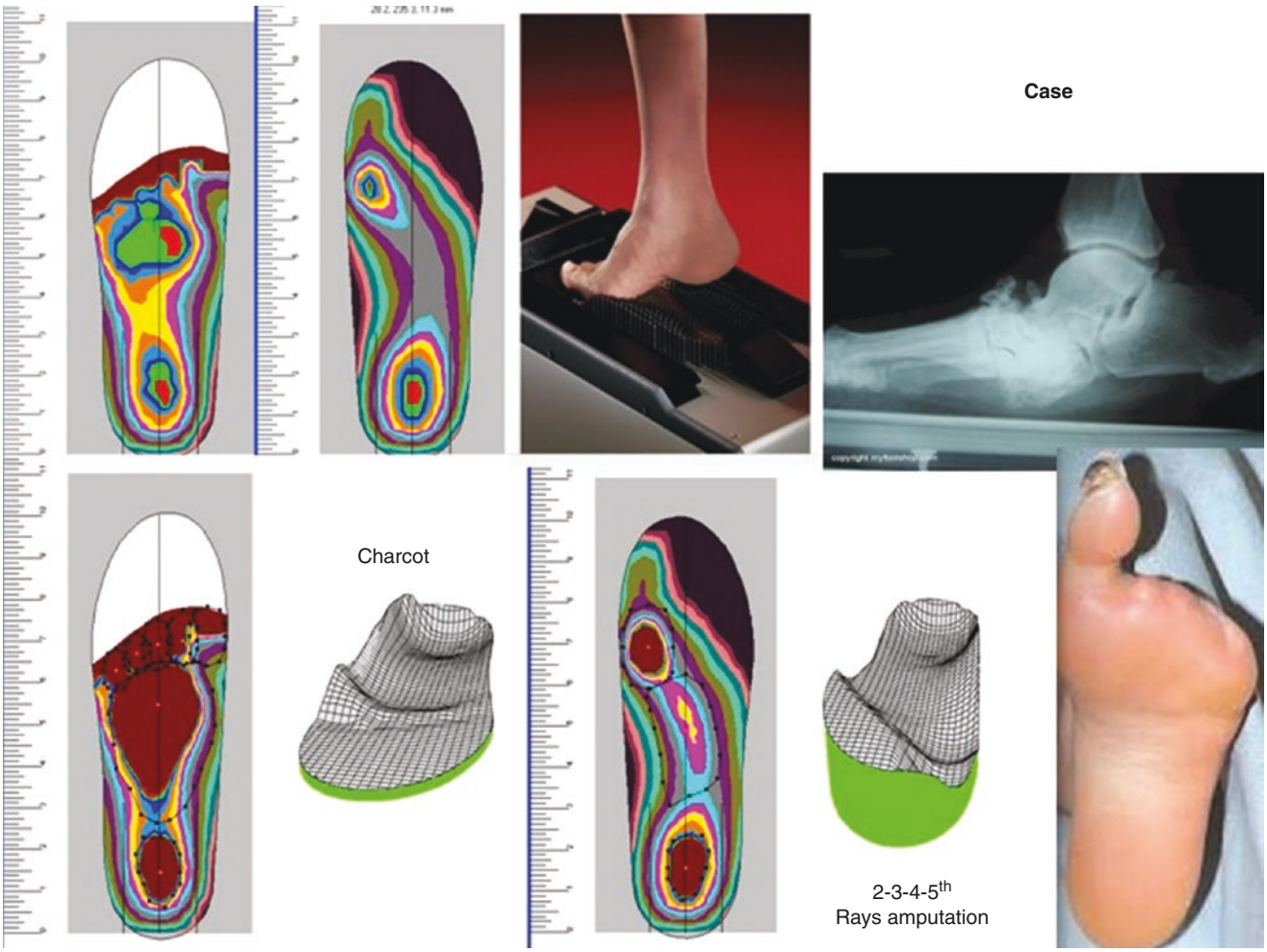


Fig. 12.3 Orthotics design for offload and support during GAIT—case: left foot with Charcot arthropathy and right foot with amputation of the 2-3-4-5 rays

diabetic foot pathology; however, intrinsic muscle atrophy does not necessarily appear to imply toe deformity, suggesting either that loss of foot muscles precedes the development of toe deformities or that intrinsic muscle atrophy is not the primary causative factor [25].

Charcot arthropathy causes gross deformation of the foot, thereby affecting functional use of the foot and causing abnormal pressure loading during walking. Peak plantar pressure in patients with Charcot arthropathy over bony prominences is higher compared with patients with a neuropathic ulcer [26]. People with minor amputations in the feet exhibit abnormal pressure loading to the same and the contralateral foot [27]. Moreover, amputation of the hallux greatly increases pressure under the MTHs [28, 29]. Increased plantar pressure is a strong risk factor for foot ulceration [13, 30]. Beyond foot deformities, callus formation as discussed below has been associated with high plantar pressures and is highly predictive of foot ulceration [31] (Fig. 12.3).

Thus, foot deformity as a consequence of neuropathy and callosities have been associated with abnormal foot loading during walking, thereby causing high plantar foot pressures. Alleviation of these high-pressure areas with accommodative footwear, including proper shoes and insoles, is necessary to reduce high pressures and to protect people with diabetes from development of ulcers.

Sudomotor Dysfunction

Sudomotor dysfunction as a result of damage of the postganglionic sympathetic nerve fibers (C-fibers) that innervate the sweat glands causes dryness of the skin of the feet. The people with diabetes who have a history of foot ulceration present a high percentage of sudomotor dysfunction compared with the diabetics with neuropathy but not foot ulcers and diabetics without neuropathy [32]. Dryness of the skin of the feet can be found in the vast majority of the diabetic patients with ulcers compared to the diabetic patients without foot ulcers and can cause fissure formation and in combination with increased plantar pressure enhances callus forma-

tion [33]. Another consequence of the autonomic neuropathy is that it causes abnormal opening of arteriovenous shunting [34], which could reduce capillary blood flow and impair oxygen delivery to the tissues, consequently diminishing arteriovenous oxygen differences and impair skin oxygenation and wound healing [35].

Changes in Tendon, Muscles, and Bones

Several investigations have looked into changes in tendon from diabetes. Plantar aponeurosis and flexor hallucis longus tendon are thicker in neuropathic patients as compared to nondiabetic controls [36]. There is a trend of increased thickness of the plantar fascia and the Achilles tendon as diabetes control worsens. These alterations result in changes in ground reactive forces, force x time integrals, and equivalent maximum loading times. Thus, for patients with foot ulcer, the ground reactive forces are larger than the nondiabetes. The equivalent maximum foot loading time is also higher in all (vertical, anterior–posterior, and mediolateral) [37]. The mechanical and metabolic muscle function is highly affected from the impaired glucose regulation due to the dependency on oxidative phosphorylation for energy production [38]. In diabetes, the muscle strength reduces and thus leads to less force in the calf and slower gait. Consequently, misfiring a reduction in neural pathways associated with muscle motor units and fiber types recruitment and deactivation will lead to mobility alterations [39, 40]. As a result, mean plantar flexion peak torque measured by an isokinetic dynamometer is reduced in diabetes [41]. The ankle and knee muscle volume and maximal isokinetic muscle strength are reduced in neuropathic diabetic patients [17]. Thus, people with diabetes and neuropathy exhibit delayed EMG responses, decreased isokinetic muscle strength and atrophy.

Muscle stiffness is a concept related to the previously described changes in muscle weakness, atrophy, and tendon thickening. Passive muscle stiffness relates to the resistance of a muscle to elongation. When a neuropathic group examined muscle stiffness in relation to strength, range of motion (ROM), and gait impairment, the researchers found that all passive peak torque variables were associated with concentric peak torque, suggesting that intramuscular structures contribute to both strength and stiffness [42, 43]. The neuropathic patient also uses passive torque for a larger proportion of total torque output. There is a substantial decrease in concentric plantar flexor peak torque, which may lead to instability when the center of mass passes anterior to the ankle joint, but there is not significant correlation between passive stiffness and ROM [43]. Thus, the muscle strength and sensation may be more related to dorsiflexion at the ankle [42]. Compared to non-DPN diabetic patients, the passive stiffness is not different. Data suggest that passive stiffness explains a significant amount of variance in walking speed. This may have clinical bearing in brace use in this population to increase passive stiffness [44].

Dynamic EMG research reported that the tibialis anterior muscle, which is responsible to control eccentrically the flattening of the foot after the heel strike phase, remained active for a longer duration of time in DPN patients compared to healthy controls [39]. The vastus lateralis muscle lags during walking and thus develops increased loads during heel strike, followed by a shorter duration of activity of the lateral gastrocnemius. An early activation of the triceps sural muscle leads eventually the forefoot and more anterior regions of the foot to make an early contact with the ground surface [39].

Under these circumstances, the temporal progression of the plantar surface and contact to the floor during stance, from the heel to the metatarsal heads and hallux, follows an abnormal route and propulsion. Consequently, high peak pressures occur on the anterior plantar areas at the initial heel contact due to its premature contact and will be added and accumulated to the forefoot that will bear loads during the toe-off phase which follows. This prolonged overload throughout the foot rollover process (at early and late stance), associated with the reduced sensitivity, would increase the risk of tissue breakdown in the diabetic neuropathic foot [39].

Data regarding the effect of diabetes on bones are contradictory. In the Women's Health Initiative Observational Study, it was found higher hip and spine bone mineral density in postmenopausal women with diabetes in comparison with nondiabetic women [45]. A small study described lower bone mineral density in the calcaneous of patients with diabetic neuropathy and in diabetic patients with mid foot deformity in comparison with nondiabetic individuals [46].

Limited Joint Mobility

The factors limiting ankle joint dorsiflexion are anatomical, physiological, or orthopedic in the nondiabetic population, but in diabetic individuals, glycosylation may be an important factor in altering the joint motion. There is a linear relation between diabetes and foot morphology deficits, especially in the presence of neuropathy.

Chronic increased tissues exposure to hyperglycemia alters their composition and functionality. Nonenzymatic irreversible glycosylation of proteins and formation of advanced glycosylation end products (AGEs) alters the mechanical properties of tissues and typically causes reduction in elasticity [47, 48]. The skin of the diabetic patients is thicker and less elastic in comparison with that of healthy people [49, 50]. Collagen bundles in the dermis of people with diabetes are thickened and disorganized as a result of irreversible glycosylation of collagen. Collagen has a low turnover rate, and the formation of AGEs damages the pro-

tein itself and reduces the ability of collagenase to remodel the collagen fibers [47]. One study found that the keratin in the stratum corneum of the diabetic foot was glycosylated compared with nondiabetic skin [51]. This may impair the capacity of the skin for the distribution of pressure.

A further consequence of AGEs formation is the limitation of range of movement in many joints of the body in people with diabetes [21]. Regarding etiology of LJM, most evidence suggest a relationship with the collagen abnormalities and nonenzymatic glycation of soft tissue that occurs in diabetes, resulting in thickening of tendons, ligaments, and joint capsules, thereby reducing tissue flexibility [47, 52–54]. LJM has been described first in the hand and can be easily demonstrated by inability to flatten the hand on a table top or by failure to approximate the palms with the fingers fanned and the wrist maximally flexed (prayer sign) [52]. The prevalence of LJM (diagnosed with a positive "prayer sign") has been reported to vary between 49% and 58% for type 1 diabetic patients and between 45% and 52% for patients with type 2 [54–56].

The first metatarsophalangeal joint (MTPJ) acts as a powerful second type lever during the body propulsion at the toe-off phase of gait. In people with DPN appears substantial biomechanical dysfunction, leading to elevated plantar pressures during gait [39]. Ulceration under the great toe has been constantly associated to a reduced ROM at the first MTPJ [8]. Patients with a history of first MTH ulceration have significantly diminished ability for dorsiflexion at the first MTPJ and at the same time increased peak plantar pressure under the first MTH; LJM of the first ray explained almost 50% of the variance in peak first MTH plantar pressure [8].

Limited joint mobility is associated with abnormally high plantar foot pressures and can contribute to foot ulceration in diabetic patients who have neuropathy [57]. Veves et al. found reduced subtalar joint mobility and increased foot pressures in people with diabetes compared to nondiabetic controls and that diabetic Caucasians had reduced joint mobility and higher plantar pressures in comparison with diabetic African Americans, suggesting that there are racial differences in joint mobility affecting foot pressures [58]. There is a significant relation between LJM at the subtalar and ankle joint and increased foot pressures in people with diabetes [41]. The maximum movements at the ankle are delayed and slowed in people with long-standing type 1 diabetes and the ROM is linearly associated with the severity of the diabetic neuropathy [59]. In the case of diabetic patients with ulcers there's a significant impairment in the ROM of the subtalar joint in comparison with nondiabetic controls and diabetic patients without ulcers [60]. In addition, type 1 diabetic people in India were found to have LJM of the foot which decreased further with longer duration of diabetes [61].

The passive and active range of motion at the ankle and first MTP joint was assessed in a cross-sectional study including people with diabetes, with diabetic neuropathy, with foot ulcer, and a nondiabetes reference group. The authors found significant reductions in both measures for first MTP joint dorsiflexion for the ulcer group vs. the reference group [62]. There might be associations between neurologic indices and forefoot deformity as well as limited mobility of the first MTP joint in people with type 2 diabetes [63]. Comparing people with type 1 and type 2 diabetes as well as healthy controls, there's a reduced joint mobility at the ankle and the first MTP joints in patients at high risk for foot ulceration as compared to those with diabetes not at high risk and the controls. In those cases, the pressure-time integrals are significantly higher in the foot-at-risk patients compared with the others and there is a strong inverse correlation between the mobility of the ankle or first MTP joints and the pressure-time integrals of the diabetic patients [64].

Another factor which influences is aging. Aging and diabetes causes a significant reduction in the plantar and dorsal flexion of foot ankle joint mobility, though after adjusting for age, diabetes specifically reduces plantar flexion only [65].

The first ulceration can be significantly detected on the foot which presents lower ankle joint mobility, and eventually the ankle joint mobility remains lower in patients with history of previous foot ulcer [65].

There are limited data on the effect of interventions aiming at improvement of ROM in people with diabetes and no data on the association with foot ulceration. Passive physical therapy in patients with foot ulceration can improve joint mobility and reduce plantar pressures [66]. More active exercise therapy must focus on the improvement of joint mobility, muscular performance, and walking speed in diabetic patients [67].

Foot ulcers are frequently healed using casts for offloading, and in addition patients are advised to minimize their level of physical activity while healing the ulcer; these two factors may compromise joint mobility. The use of N-phenacylthiazolium bromide, a substance that cleaves collagen crosslinks, could help in combination with passive mobilization, to improve joint mobility [68, 69].

The people with diabetes develop changes in weight bearing during ambulation, due to limited joint mobility that occurs mostly at metatarsophalangeals and subtalar joints. The transfer requires postural adjustments and is crucial to facilitate the walking as well as to maintain the balance during dynamic tasks. The weight distribution between the lower limbs (weight shift) is an important parameter and can be measured in a quite bipedal stance,

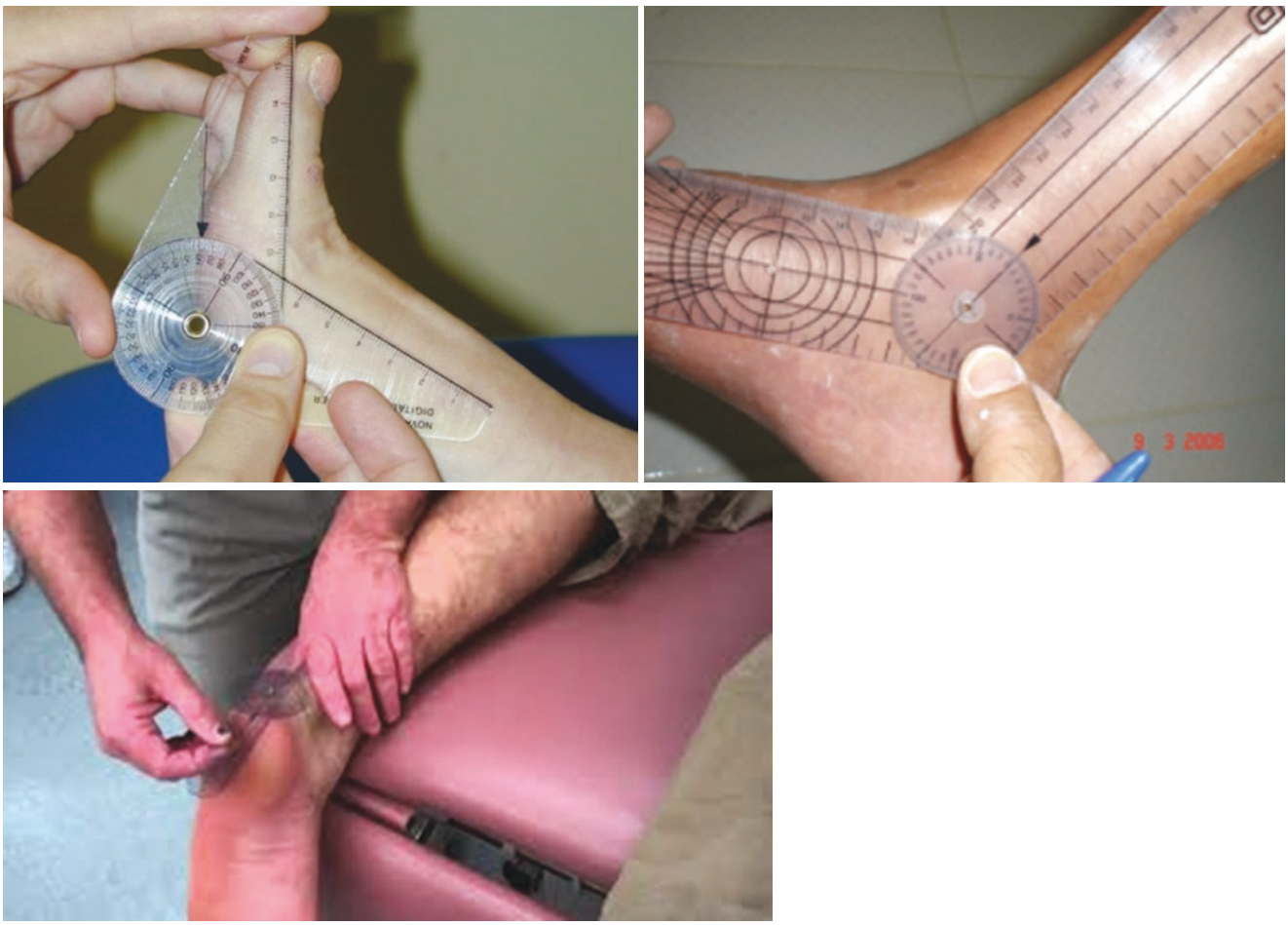


Fig. 12.4 Range of motion measurements with a goniometer, for the ankle—subtalar and 1st metatarsophalangeal joints

using force and pressure platforms (Fig. 12.5). Patients with DPN have uneven weight distribution in each leg, especially in the presence or history of ulcers. The uneven weight distribution is notable when the difference between the legs exceeds the 5–8%. In this case, there are usually anatomical differences, like the leg length that may require a customized foot orthotic. In the case of no leg length difference, other functional reasons, such as affected trunk alignment (e.g., scoliosis), weakness of gluteus medius, pelvic and core musculature shortening and/or weakness, hip joint capsule and ligaments stiffness, and morphological differences between the feet (like the high of the foot arch), may be the main cause.

The active and passive range of motion (ROM) of the main foot joints, which act dynamically during the stance phase of gait, such as the first metatarsophalangeal joint (1st MTPJ) and the ankle and subtalar joints, can be measured using special goniometers (hand held or digital) (Fig. 12.4).

In summary, diabetes may exacerbate reduced joint mobility that typically occurs with aging and many studies found an association with the severity of diabetic neuropathy and previous foot ulceration. However, it is important to note that most of the evidence on the relationship between LJM with foot ulceration came from cross-sectional studies. There is need for prospective studies to examine the relationship between LJM with foot ulceration as well as to examine whether interventions aiming at increased joint mobility affect incidence of foot ulceration.

Fat Pad and Plantar Fascia Changes

Many studies described atrophy, relocation, and changes in absorption and shear properties in fat pads for people with diabetes [25, 70]. Nondiabetic people in comparison with diabetic patients with and without ulcers have thicker fat pads at the heel and first and second metatarsal heads [71].

The submetatarsal fat pads appear significantly thinner and the subphalangeal fat pads significantly thicker in the neuropathic patients with deformity compared with neuropathic control patients, suggesting thinning and distal displacement of the fat pad due to the contracture of the digits [25]. The reduced fat pad is associated with bony deformity and increased peak plantar pressures [71]. Rheumatoid arthritis and diabetes can cause foot deformities, reduced fat pad, and abnormally high pressures under the feet. In people with diabetic neuropathy and in people with rheumatoid arthritis with similar foot deformities plantar pressures are evenly high, but only the diabetic patients with neuropathy develop foot ulcers [71]. This emphasizes the importance of the loss of sensory awareness in the pathogenesis of diabetic foot ulceration, and suggests that high pressure alone is not a direct cause of ulceration [72]. Reduction in fat pad can be so prominent in some patients with claw toe deformity that the condyles of the MTH can be palpated under the skin. In addition, callus can develop at the tips of the clawed toes due to the high concentrated pressure.

Plantar fascia is a critical element in the transmission of force through the toes. The diabetic patients present increased thickness of plantar fascia. Thus, in association with the mobility of the MTP joint and the forces expressed under the metatarsal heads, the patients with foot ulcers compared with nondiabetic have a significantly reduced ROM at the MTP joint and increased vertical and mediolateral forces [73]. Limited evidence suggests that rupture of the plantar fascia after repetitive chronic stress can contribute to the development of toe deformities [74].

These data suggest that fat pad is reduced in diabetic patients with neuropathy and foot deformities and it is associated with increased plantar pressures. The thickness of the plantar fascia is increased in people with diabetes and it is associated with reduced ROM and high plantar pressures.

High Plantar Pressures

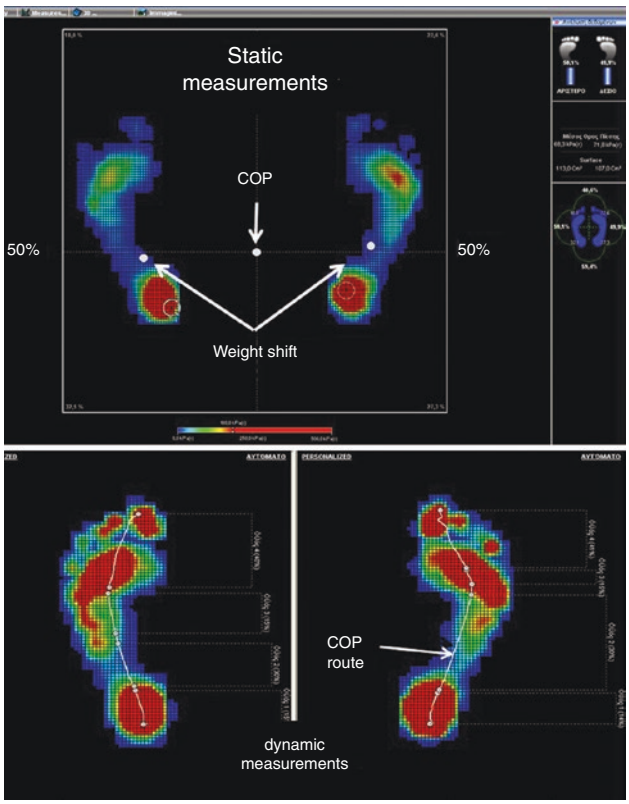
As described before, a number of foot alterations in people with diabetes cause increased plantar pressures. Early observations described that most neuropathic ulcers occur on the toes (39%), the hallux (30%), and the metatarsal heads (24%) [75]. These areas are of principal concern in understanding the causes of elevated pressure. Veves et al. described that a value of over 1000 kPa during barefoot walking is required for the development of an ulcer over a 2.5-year follow-up period; in contrast no ulcer developed in patients with lower plantar pressures. These ulcers developed in high-pressure areas like the MTH [13]. A threshold of 700 kPa has a high percentage sensitivity and specificity for the prediction of foot ulceration [76] and peak pressures over 875 kPa seem capable for prediction of foot ulcer development [77].

The isolated measure of peak pressure does not incorporate a time dimension. It is important to examine the role of pressure time integral and plantar stress in the development of ulcer. Diabetic patients with neuropathy and ulcers have higher peak forefoot pressures and pressure time integrals than diabetic patients without neuropathy [78].

Patients with recurrent ulcers are less active and compile less daily stress, expressed as the product of mean daily strides and forefoot pressure-time integral, than diabetics without neuropathy and nondiabetic controls. The patients with a history of ulcer may be more susceptible to plantar tissue injury even at relatively low levels of activity and cumulative tissue stress [79]. Although individuals with diabetes who develop ulceration have a lower overall activity than their counterparts with no ulceration, activity levels in ulcerated patients have a high degree of variation, which increases further a few weeks prior to ulceration [80].

Barefoot walking is believed to be a principal cause of foot ulceration. Some patients may protect their feet adequately in footwear throughout the day, yet ulcerate because of barefoot walking at home [9]. The type of shoes worn is important; thus, walking in shoes with leather soles is almost equivalent to walking barefoot while walking in simple sport shoes (trainers) can reduce pressure up to 50% [9]. Pressure reduction strategies alone do not have the greatest impact on preventing reulceration. Therapeutic footwear and orthotic devices reduce drastically (by 50–80%) plantar pressures but many (26–42%) of these patients reulcerate within 12–18 months [81, 82]. Observational studies have shown that only 22–29% of individuals wear their prescription footwear for 80% of the daytime [83]. Patients who adhered to wearing custom-made footwear are less likely to reulcerate in a mean follow-up period of 18 months [84]. It is believed that people with diabetes view their homes as safe zones and may not wear the prescribed footwear, despite taking over 50% of the daily steps at home [9]. An additional factor that affects compliance is the perceived benefit of the footwear in prevention of foot ulcer [85].

Another important issue is the contribution of pressure gradient in the pathogenesis of foot ulcers. Pressure gradient is defined as the spatial change in plantar pressure around the peak pressure location. The mean peak pressure gradients develop higher in the forefoot than in the rearfoot, whereas the mean peak plantar pressure is only 36% higher in the forefoot than in the rearfoot. Moreover, the peak pressure gradient forefoot-to-rearfoot ratio is nearly two times greater than the peak plantar pressure forefoot-to-rearfoot ratio. Thus, the peak pressure gradient appears to provide additional information about the stresses experienced by the soft tissues of the foot, especially in the forefoot [86]. Additionally, the maximal shear stress is by 1.29 times higher and closer to the sur-



Kinetic Data :

- Pressure distribution around the plantar area during quite bipedal stance
- COP orientation on the transverse plane during quite bipedal stance
- Body Weight % distribution between lower limbs during quite bipedal stance (weight shift)
- COP kinetic data during quite bipedal stance (static balance)
- Pressure distribution around the plantar area during the stance phase of GAIT (dynamic)
- COP kinetic data during the stance phase of GAIT
- Spatio-temporal data during the stance phase of GAIT

Fig. 12.5 Static and Dynamic measurements using a foot pressure platform (pedobarograph); *static* measurement data: Pressure distribution around the plantar area; Center of Pressure (COP) orientation on the transverse plane; The Body Weight % distribution between the legs (weight shift); COP kinetic data (COP sway velocity and area)—*dynamic* measurement data: Pressure distribution around the plantar area during the stance phase of GAIT; COP route from heel strike to toe-off; spatiotemporal data during ambulation, including the distances (cm) for Gait—step—stride and the lateral width between the legs and the relevant times (sec)

face in the forefoot compared to the rearfoot, with significant correlations between maximal shear stress and peak pressure as well as maximum pressure gradient [87].

Charcot foot with a "rocker bottom" deformity is associated with increased plantar pressure [88]. Many persons with this deformity develop recurrent ulcers over the bony prominences. The deformities associated with Charcot affecting less commonly the ankle or rearfoot are often multiplanar, resulting in sagittal, frontal, and rotational malalignment [89]. In addition, shortening of the limb often occurs from collapse of the distal tibia, talus, and calcaneus [90]. These deformities also result in alterations in the biomechanics of the foot. For example, a varus ankle or rearfoot results in increased lateral column plantar pressure of the foot, predisposing the patient to lateral foot ulceration. Collapse of the talus, secondary to avascular necrosis or neuropathic fracture, further accentuates these deformities and contributes to a limb-length inequality [90].

Recording and Evaluation of the Pressures Around the Plantar Area

Plantar pressure measurement is being used in both research and clinical practice to compare gait patterns of different clinical groups and to evaluate the effect of footwear, orthotic and surgical interventions [91].

When someone stands upright and much more when ambulates or run, the foot structures receive high pressures. As pressure is defined as the quotient of the force (including weight and muscle force) exerted on the foot by a surface that receives this power. The pressure is expressed in kg/cm^2 or in kPa (kilopascals). Since a typical man's size −10 foot has a total area of approximately 130 cm^2, the average pressure under the foot is of a 100-kg person would be 0.77 kg/cm^2 or approximately 75 kPa. It is estimated that plantar pressure is by 40% greater during running than those encountered during walking. Moreover, the pressure that applies on tissues during walking under a callus or a scar from a healed ulcer can be almost 10–15 times higher compared to healthy people [9].

Pedobarographic Evaluation

For the recording and the evaluation of the plantar pressure distribution, there are in the market special platform like apparatus (pedobarographs or FPPs), which consist of digital sensors and calculate the force per square area (N/cm^2) (pressure in KPa). The amplitudes of the applying forces attributed analogously through a RGB color scale (with the highest pressures draw red and the lowest draw blue). As a result, a full image of the loading plantar area of the foot can be acquired, with the specific coloration based on the recorded pressures (Fig. 12.5). For the pressure distribution the subject adapts an upright standing (static) position (usually a quite bipedal stance), barefoot for some seconds.

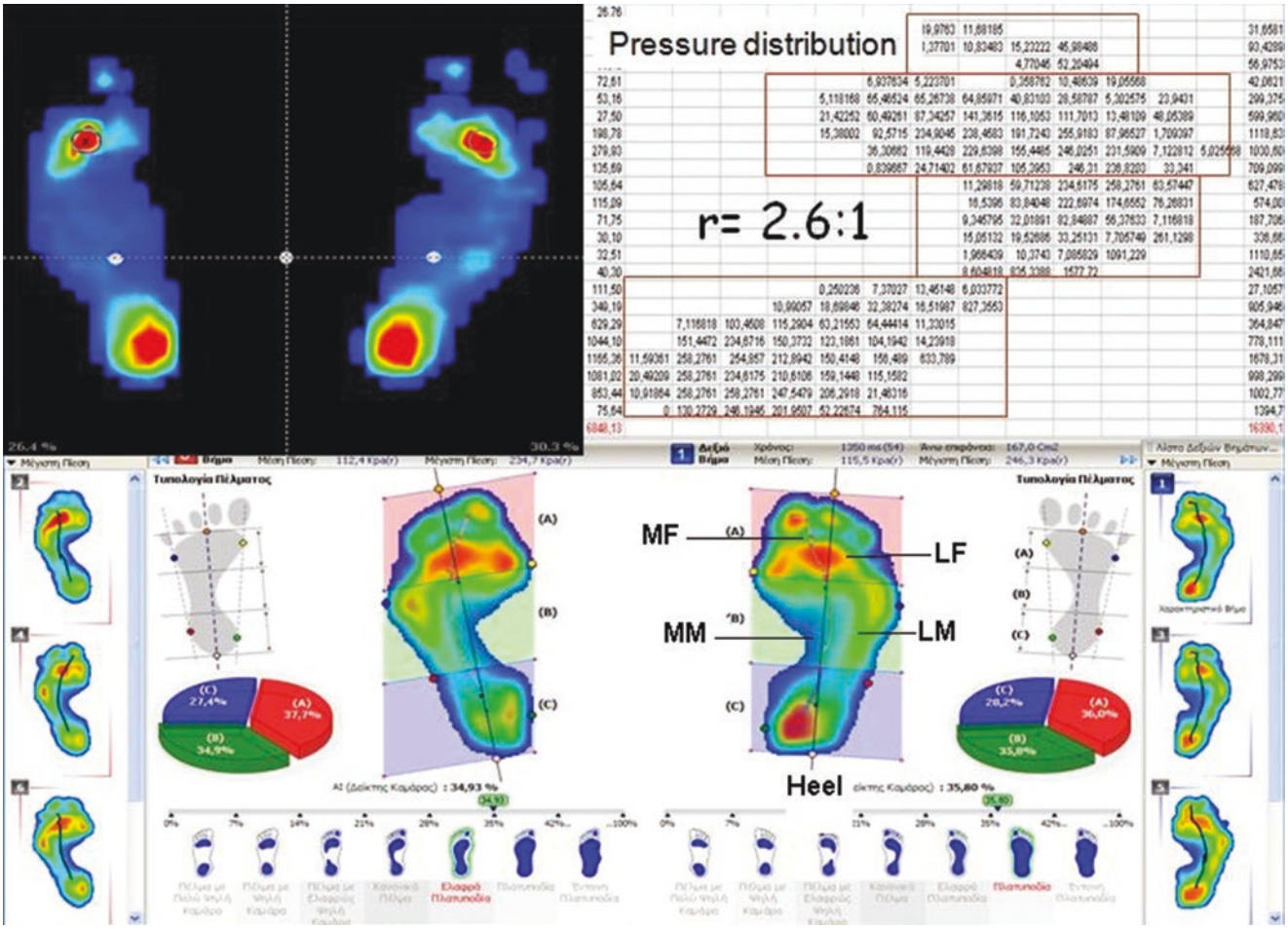


Fig. 12.6 Pressure distribution on the foot plantar area. The five segments we can divide the foot plantar area using the Bowen's model, MF: medial-forefoot; MM: medial-midfoot; LF: lateral-forefoot; LM: lateral-midfoot and Heel

Most Common Foot Deformities and High Risk Pressure Distribution

The foot structure is dominant in predicting peak pressure [13, 26] (Fig. 12.6).

A foot can be characterized as cavus if the middle third of the footprint cover less than the 2/3rd of the forefoot print's width; planus (flat) if the width of the middle third of the footprint exceeds 1/3rd of the full foot width. The heel deviation (eversion—inversion) can be evaluated by comparing the Helbing line (drawn along the Achilles tendon) with the vertical one. A valgus deviation higher than 38° is considered as a valgus heel (eversion—peak pressures locate at the medial aspect of the heel). A varus deviation is considered as a varus heel (inversion—peak pressures locate at the lateral aspect of the heel) [15] . Additionally, a Hallux valgus is defined as a deviation of the great toe toward the lateral side of the foot with a prominence developed over the medial side of the first metatarsal head. In this case, the COP route during the rollover process is highly affected and lead to an overload of the lateral metatarsals and also instability during the toe-off phase of gate [15] (Fig. 12.7).

In the case of cavus diabetic foot, there are higher peak and mean peak pressure values on the forefoot area, when compared with people with normal foot. This phenomenon has also been related to submetatarsal pad displacement and/or increased stiffness in non-DPN and DPN patients, as well [15, 92].

In case of abnormal alignment of the foot with an uncompensated forefoot varus or forefoot valgus (inverted or everted forefoot), high pressures appear located at the first or fifth metatarsal head, respectively. For the same dynamic reasons, an inverted heel and load is associated with lateral heel high pressures, whereas an everted heel position gives medial heel high pressures [93].

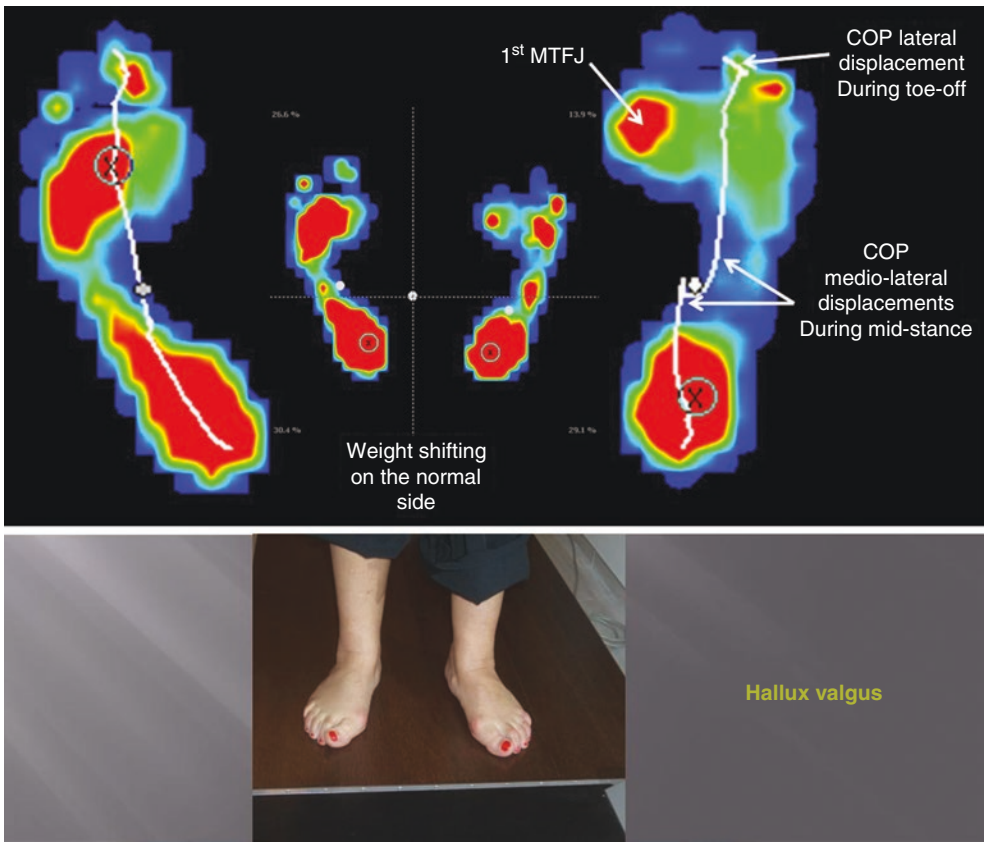


Fig. 12.7 Case: Hallux valgus, static and dynamic measurements. Weight shifting on the normal side. A prominence developed over the medial side of the first metatarsal head. Overload of the lateral metatarsals and instability during the toe-off phase of GAIT

The Spatiotemporal And Kinetic Parameters During Ambulation

The spatiotemporal parameters are reduced in DPN patients compared to non-DPN diabetic and healthy people. The DPN-related changes in the lower limbs lead to gait variations and deficits; individuals with DPN walk slower than healthy people and have smaller stride length. Studies reported also, slower walking speeds in the DPN patients compared to non-DPN patients, with a longer percentage duration in the stance phase of gait and a longer stance time [39]. One hypothesis is based on the dynamics of the kinematic chain of the limb, where the force generation at the hip, knee, and ankle increase significantly for both flexion and extension moments in patients with DPN [39]. The excess of hip flexion is also another compensatory mechanism to increase stability in the gait strategy of DPN patients, adjusting the impaired ankle dorsiflexion [39].

Kinetics of the Diabetic Foot During Gait

The forces generate during heel strike and toe-off phase of gate in patients with DPN have lower magnitude compare to non-DPN diabetics and healthy people [94]. The first maximum support moment (combination of extensor moments at hip, knee and ankle) and the mid stance minimal support moment appear elevated in DPNs compared with non-DPN diabetics (this suggests that combined forces at the hip, knee, and ankle during the stance phase are greater in DPN patients compared to the others); however the second maximum support moment seems slightly higher in the non-DPN diabetic patients when compared to DPN patients [94, 95]. Additionally, people with DPN generate greater knee flexion moment compared to non-DPN diabetic and healthy people [94], probably because knee flexion might be an important compensation strategy in those with DPN, as the motor component of DPN manifests in a stocking and glove distribution and affects the distal joints first [39]. They also generate greater hip extension moment and reduced hip flexion moment [95].

The ground reaction force (GRF, Z-axis) during the initial contact (heel strike) and in some cases during toe-off are higher in DPN patients compared to both non-DPN diabetic and healthy people due to the neurological deficit and reduced proprioception [39]. The forward peak and backward peak of the anteroposterior ground reaction force (Y-axis) component appear reduced during the stance phase. DPNs with previous neuropathetic ulcer showed a significant increase of the mediolateral stress (X-axis), especially under the metatarsals [91] (Fig. 12.8).

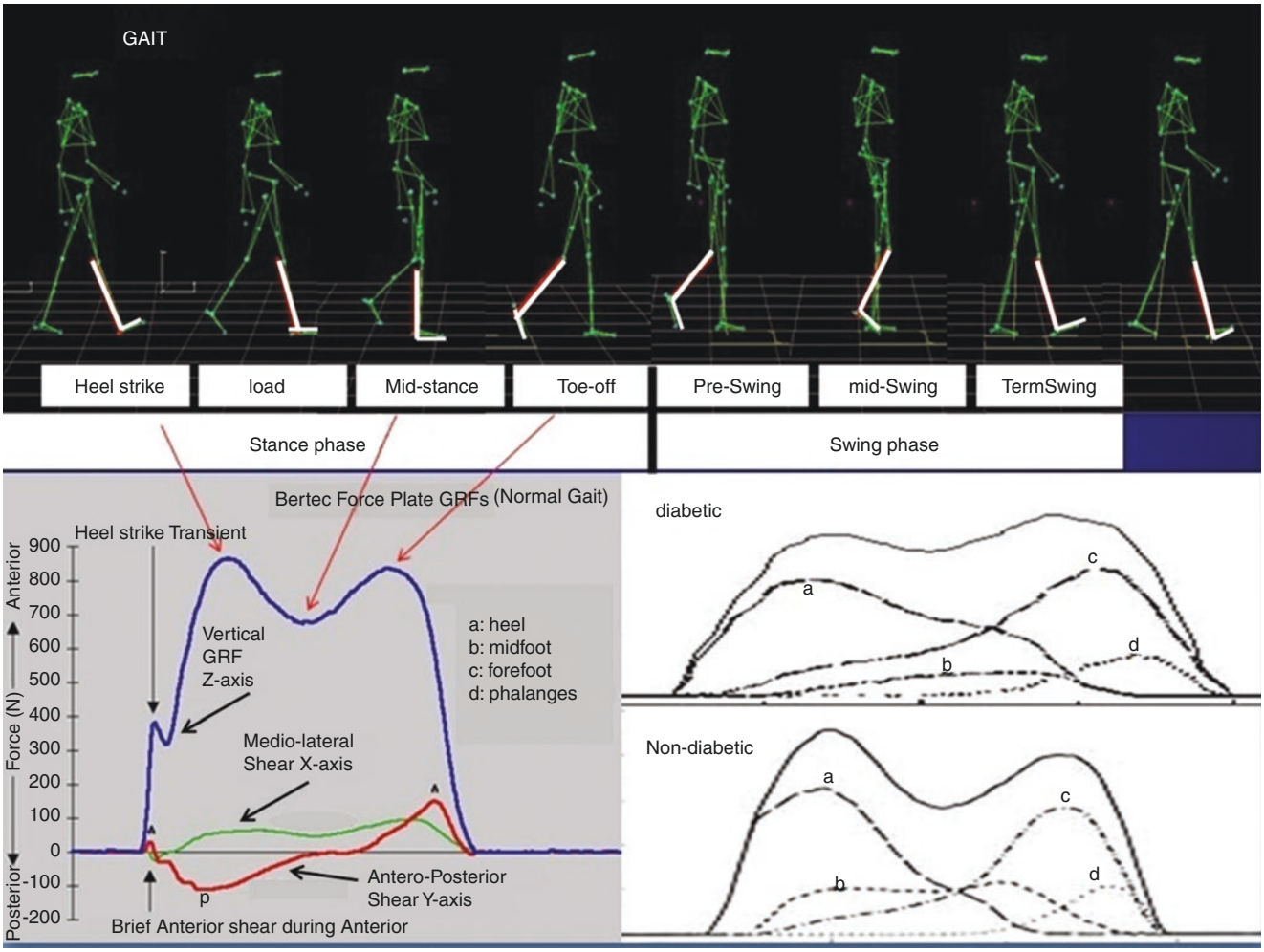


Fig. 12.8 Ground reaction forces during the stance phase; Case: vertical GRF (Z-axis) differences between a diabetic neuropathic and a non-diabetic stance phase; (**a**) heel load, (**b**) midfoot load, (**c**) forefoot load, and (d) phalanges load. During the diabetic stance, the midfoot load (**b**) is reduced and short timed and thus we observe a quick transition and overload on the forefoot

In the presence of ulcers or ulcers history on the foot, DPN patients would continue to demonstrate similar abnormal lower limb biomechanical characteristics, like reduced spatiotemporal parameters such as speed of walking and stride length, restricted kinematics, delayed muscle activations, and altered forces (kinetics), which may contribute to elevated plantar pressures during gait and entrap the person into a vicious cycle of repetitive pathology [96].

Concisely, the four biomechanical diversifications for patients with DPN compared with the normal gait are abnormal spatiotemporal outputs, such as speed of walking and stride length, restricted kinematics (movement patterns), altered kinetics (altered forces), and elevated plantar pressures on the ulcerated and non-ulcerated foot.

Plantar Callus

Plantar callus develops mainly at areas of high vertical pressures like the MTH and hallux [97]. Callus acts as a foreign body and acts to elevate further pressure resulting in positive feedback for the production of callus. Callus, if left, results in injury to the underlying tissues in the presence of loss of protective sensation [97], while its removal reduces plantar pressure and prevents ulcers [98]. Delbridge et al. suggested that the initial event in the formation of a neuropathic plantar ulcer is the development of callus [31]. Then tissue injury occurs under the callus and a cavity is formed that is filled with blood (hemorrhagic callus or pre-ulcer). This cavity enlarges with further walking until it causes a rupture of the skin surface forming an ulcer. Prospective data demonstrated that the

presence of plantar callus was highly predictive of subsequent ulceration and callus is recognized as a "high-risk" factor for foot ulceration [99].

Previous Foot Ulceration

Previous ulceration is a leading risk factor for future ulceration. In addition to the risk factors a patient has to develop the first ulcer, altered mechanical properties of the new tissue generated during wound healing may increase further the risk [100]. Actually, little is known about the properties of tissue formation during wound repair [101]. It is believed that the hard scar tissue that is formed may act in much the same way that callus act by transferring increased pressure to the underlying soft tissues. Indeed, Murray et al. demonstrated that a history of previous ulceration offered the highest relative risk (56.8) for reulceration compared to a much lower relative risk (11.0) for an ulcer developing under an area of callus [31]. Contrary to what was previously thought, patients with active ulcers have reduced plantar pressure compared to those with DPN without ulcers, probably because they adapt a more "conservative and consciously depended gait strategy." Of course, that will eventually affect and overload the contralateral foot.

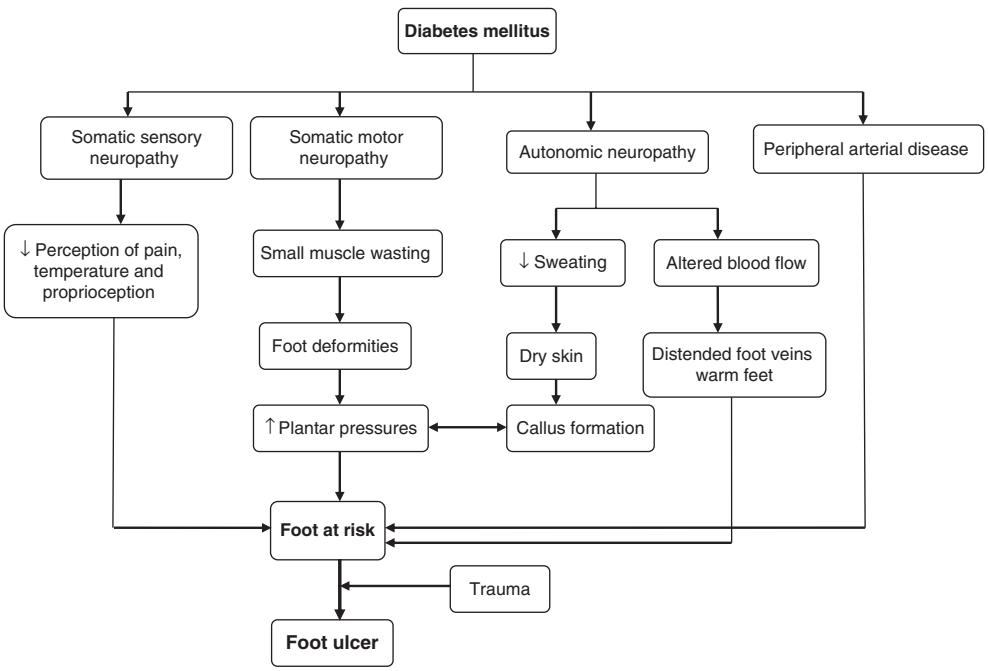
Development of Diabetic Foot Ulceration Due to Physiological, Habitual, and Biomechanical Alterations

The breakdown of the diabetic foot has traditionally been considered to result from peripheral neuropathy and peripheral vascular disease. As discussed before, other contributory factors such as limited joint nobility, high plantar pressures, deformities, autonomic neuropathy, and psychological factors are implicated. The role of peripheral vascular disease and peripheral neuropathy will be discussed in detail in Chaps. 3 and 4, respectively.

Peripheral vascular disease itself, even severe, does not cause foot ulceration. Minor injuries and/or infections will increase the demand for blood supply beyond the circulatory capacity. In the presence of peripheral vascular disease, the failure of the circulating blood rising in the region causes ischemia and hypoxia, resulting in tissue destruction and development of an ischemic ulcer or gangrene [102] (Table 12.1).

Many studies have confirmed the role of diabetic neuropathy in the etiopathogenesis of foot ulceration. Cross-sectional data confirmed that neuropathy is present in up to 90% of foot ulcers alone or in combination with peripheral vascular disease [103]. The EURODIALE study included 1229 consecutive persons presenting with a new foot ulcer

Table 12.1 The pathway to foot ulceration



Modified from reference [101], with permission

in 10 European countries. At baseline, peripheral vascular disease was diagnosed in 49% and diabetic neuropathy in 86% of the participants [104]. Prospective data demonstrated that neuropathy assessed by vibration perception over 25 Volts was associated with a sevenfold annual increase in the risk of ulceration in a 3-year follow-up period [105, 106]. Another large prospective study described that beyond other factors including previous ulceration, foot deformities, reduced pedal pulses, neurological modalities like insensitivity to the 10 g monofilament, and a neuropathy disability score ≥6 were independently associated with almost 2 times higher risk of foot ulceration over a 2-year period [23]. The above data clearly suggest that neuropathy is a strong risk factor for foot ulceration. Yet, the neuropathic foot does not ulcerate spontaneously and it is the combination with other factors that lead to skin breakdown. The pathway neuropathy contributes to foot ulceration is depicted in Table 12.1**.**

Reiber et al. used the Rothman model for causation and applied this to ulceration [107]. The model is based on the concept that a component cause (neuropathy or peripheral vascular disease) is not sufficient itself to lead to ulceration, but when component causes act together they may result in a sufficient cause which eventually leads to ulceration. They showed that the commonest triad of component causes present in 63% of ulcers was neuropathy, foot deformity, and trauma [107]. Trauma could be intrinsic, such as repetitive stress from high pressure and/or callus, or extrinsic such as from ill-fitting footwear rubbing on the skin or an object inside the shoe (e.g., drawing pin and pebble). Examples of two-component pathways to ulceration are: neuropathy and mechanical trauma caused by ill-fitting footwear or callus or deformities; neuropathy and thermal injury caused by hot water or heating devices; neuropathy and chemical injury caused by "corn-cures" [23]. As trauma plays a key role in the pathogenesis of ulceration, it is important to try to minimize the risk of trauma in patients with loss of protective sensation implementing preventative care such as the provision of appropriate foot care, education, and referral for podiatry treatment.

Most ulcers in diabetic patients with neuropathy occur at sites of high plantar pressures or stress [9, 11, 13]. High pressures are not usually found in healthy people and would result in pain during ambulation for an individual with adequate sensation. For example, patients with gross foot deformities from rheumatoid arthritis do not develop ulcers because they can feel the pain and adjust their gait to avoid bearing load on a painful area [72]. The repetitive application of high pressures and stress to the same area, usually overlying bony prominences, in the presence of neuropathy cause tissue damage that begins close to the bone [9, 88]. Callus develops as a result of increased pressure at the surface in order to protect the skin from further damage; in the presence of neuropathy, it is not perceived by the patient who continues his activity and if callus formation becomes excessive it will contribute to higher pressure [90]. Foot deformities, reduced LJM, and fat pad are usually responsible for the excessive pressures. The tips of the clawed toes can themselves be locations of ulcers due to concentrated pressure [108, 109]. In addition, healing of plantar ulcers is prevented as long as patients keep walking on their foot wounds, thus highlighting the key issue of mechanical offloading. Thus, excessive and/or repetitive pressures appear to be the main causative factor for development of skin breakdown.

There are three main mechanisms that account for the occurrence of these pressures: (1) increased duration of pressures; (2) increased magnitude of pressures, or (3) increased number of pressures [110]. The first mechanism includes relatively low pressures applied for a long period of time causing ischemia. Prolonged ischemia leads to cell death and wound formation, as has been demonstrated in a classic experiment [111]. It should be noted that some regions of the plantar tissue become ischemic during standing and walking. Plantar pressure in the forefoot during walking is at least 30 times higher than the systolic blood pressure in the arterial bend and even higher at the arterioles, implying that blood flow will be occluded during gait cycle [13]. Recovery from this transient ischemia can be affected by changes in microcirculation caused in the early course of diabetes, nutritional status and arterial disease [112].

High pressures took a relatively short time to cause ulceration whereas low pressures took a relatively long time. Thus, ulceration can develop at very low pressures, but may take a few days to occur. This type of offending pressure and resulting ulcers can occur with ill-fitting footwear, improperly fitted orthotics, or prolonged resting of a heel on a bed or footrest [113]. The second mechanism of tissue injury includes high pressures acting for a short-time period. This injury only happens if a large force is applied to a relatively small area of skin; this happens, for example, if a person steps on a nail [111]. Alternatively, a "foot slap" may also conform to this mechanism. A "foot slap" indicates a reduced deceleration of the forefoot after heel strike caused by weak dorsiflexion muscles [113]. It is therefore suggested that control of the velocity of the forefoot descending after heel strike by using ankle-foot orthosis could possibly help in prevention of diabetic foot ulcers [114]. The third mechanism of injury comes from repetitions of pressure, which would lead to an equivalent syndrome of mechanical fatigue [114]. Mechanical fatigue is defined as failure of a structure or biological tissue at a submaximal level to maintain integrity resulting from repeated bouts of loading. This type of injury seems to occur in the insensitive skin and subcutaneous tissue of the neuropathic foot [114].

Thus, not only the magnitude of the plantar pressure is important in causing foot ulceration but also several other

factors such as the rate of increase of pressure, duration of high pressure, and the frequency of applied pressure to the skin should be taken into account. In addition, although foot pressures may be high during a barefoot pressure assessment, it is important to keep in mind that it is the combination of footwear, life style factors, tissue characteristics, foot pressures, and level of physical activity, which contribute to the development of foot ulceration. The effect of physical activity on development of foot ulceration is an area which deserves further exploration.

Another important issue in foot injuries is footwear. While appropriate footwear can be of great benefit in preventing ulcers, incorrect footwear can actually cause ulceration [9]. It is not uncommon for the patients with loss of protective sensation to wear a pair of shoes three sizes too small because a very tight fit stimulates the pressure nerve endings and this is interpreted as a normal fit [9, 108]. The EURODIALE study described that the majority of ulcers (52%) were located on the non-plantar surface of the foot and the most frequent ulcer site was the dorsal or interdigital area of the toes (32%), while the classic plantar forefoot or midfoot ulcer was present in 22% of the patients [98]. This finding implies that a large number of ulcers develops as a result of poor footwear and emphasizes the need for footwear education for the prevention of foot ulcers.

Kinetic Control and Diabetic Peripheral Neuropathic Patient's Balance and Mobility

The musculoskeletal kinetic control is the process through which someone is able to fulfill the locomotor requirements in any given task or day live activities, like gait and balance, adapting and synchronizing his neuro-musculo-skeletal system components. To manage activities of daily life the Central Nervous System coordinates postural components which stabilize the body and the prime mover components which relate to the particular motor task on basis of an internal representation of body posture, including a model of body geometry, body kinetics, and body orientation with respect to gravity. Thus, to function in daily life, an individual must be able to maintain and adopt various postures, react to external disturbances, and use automatic postural responses that precede voluntary movements.

For the diabetic neuropathic person, impaired balance is one of the most common risk factors associated with falls [38]. Diabetic neuropathy may compromise postural stability since the integrity of the Kinetic Control and its adjusted proprioceptive system, is a critical factor for postural stability. The balance ability is a complex outcome that requires the integration of multiple sensorimotor and cognitive processes [96]. DPN has long been considered the most dominant mediator between diabetes and falls because of reduction in lower-limb somatosensation, thus reduces the ability to detect changes in balance and make appropriate adjustments to avoid a fall [38, 115]. Furthermore, age-related deterioration in those systems can disrupt the ability to maintain balance [38, 96].

The kinetic control and balance are based on three sensory systems (somatosensory, visual, and vestibular), which contribute the sensory information required for balance control. Peripheral neuropathy affects the sensory, motor, and autonomic components of the nervous system and presents as a loss of sensation, intrinsic foot muscle atrophy, and foot skin anhydrosis [39]. That's a long-term result of the affected microcirculation associated with poor glycemic control in type 2 diabetes, which eventually compromises those systems.

The somatosensory system provides information about the position and motion of body's segments in relation to each other and the support surface by using proprioceptive (joint position/kinesthesia) and cutaneous (touch and vibration sensitivity) inputs. Long-term hyperglycemia can lead to a progressive deterioration of sensory nerve fibers in the somatosensory system and eventually to DPN. Muscle spindles which provide rapid information about changes in muscle length, and the Golgi organs of tendons which sense changes in muscle tension, are affected and the cutaneous mechanoreceptors which provide information about vibration and pressure sensations, as well [38]. Thus, the greater motor error at the joints (higher g forces) leads to motor control deficits.

The visual system provides information about the environment and body orientation. Long-term hyperglycemia affects the circulatory system of the retina and can lead to diabetic retinopathy. Older adults with type 2 diabetes and reduced contrast sensitivity have been reported to be 1.41 times more likely to fall as compared to older adults without type 2 diabetes [38].

The vestibular system provides information about head position (thus, adjusting body posture), spatial orientation, and spatio/temporal input, especially the sense of velocity/acceleration. Long-term hyperglycemia causes inflammation and reduced sensitivity of the highly active metabolic vasculature in the inner ear [115]. Vestibular dysfunction has been reported to be 2.3 times more likely in those with diabetes than in those without diabetes [115]. The vestibular apparatus in the inner ear (labyrinth) and nerve synapses is highly vascular and the poor supply of oxygen decreases the autonomic and somatic reflexes.

Eventually, deterioration of one or more of those sensory systems reduces the peripheral information and the ability of the central nervous system to compensate and organize the kinetic strategy of the individual and that affects balance and increases fall risk. Therefore, it is critical to assess not only somatosensory functions related to DPN but also visual and

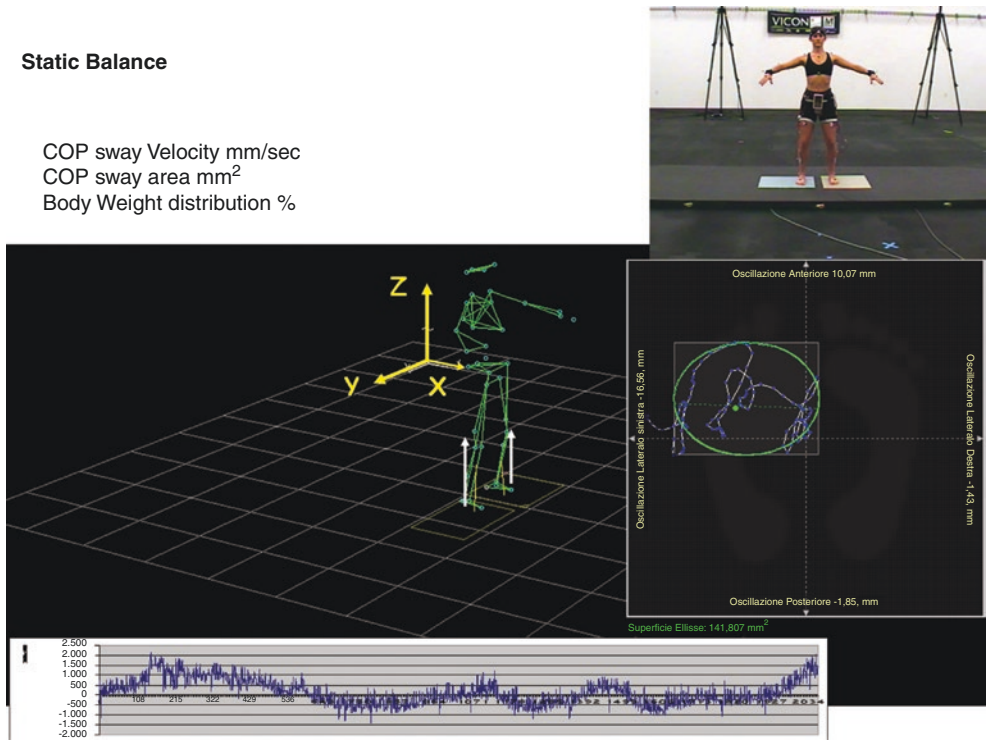


Fig. 12.9 "Good" static balance prerequisites: minimum COP sway velocity mm/sec and COP sway area mm^2 and even body weight distribution %

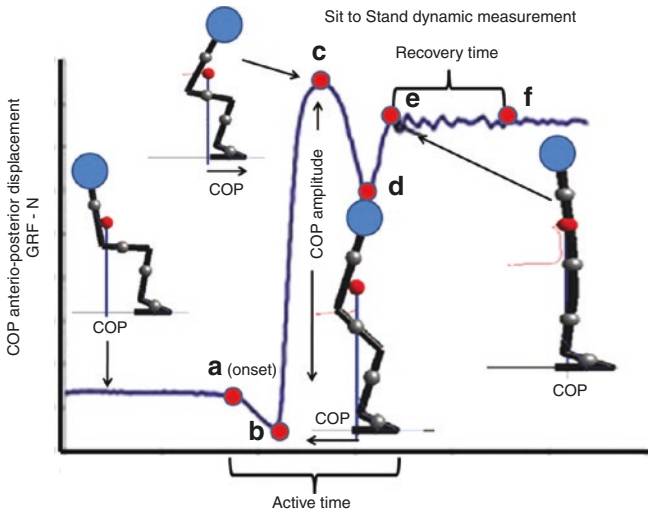


Fig. 12.10 "Good" dynamic balance prerequisites: Case: sit to stand; maximum COP anteroposterior, latero-lateral, or diagonal displacements (amplitude in mm) and minimized corresponding times (sec) for both the active (**a–e**) and recovery (**e–f**) phases

vestibular functions, which can contribute to impaired balance and falls [38].

The effect on postural stability* during stance and gait is very detrimental, with highly negative influence on mobility, and thus quality of life [114, 115]. The evaluation of the static and dynamic balance is usually based on the interpretation of center-of-pressure (COP) measures using a dynamometric platform (force-plates, pedobarographs, etc.) [115] (Figs. 12.9 and 12.10).

Many studies have reported that DPN patients are less stable than non-neuropathic diabetic and healthy people and also compare to those individuals affected by neuropathy in the asymptomatic stage, as they show more distant COP stability points (increased sway) and remain on a stable condition for a shorter time [114, 115]. Both static and dynamic balance ability are affected by the DPN and it is important to be assessed in combination with the gait kinematic and kinetic characteristics.

**[Postural stability or Balance is the ability to maintain or move within a weight-bearing posture without falling. Static balance (steadiness) and dynamic balance refer to the ability to maintain a given posture with minimal sway of Center of Pressure (COP) and to move within a given posture without loss of balance control, respectively]*

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Cell Therapies: New Frontier for the Management of Diabetic Foot Ulceration

13

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Abstract

While stem cells hold great potential to improve existing therapies for diabetic foot ulcers, their promise has not been fully exploited. There is a critical need to further develop existing sources of adult stem cells known to improve DFU healing and to test novel, replenishing sources of pluripotent stem cells (iPSCs) that may overcome impaired wound repair when delivered to DFUs. This chapter summarizes the capacity of multiple adult stem cell sources, including bone marrow-derived mesenchymal stem cells, hematopoetic stem cells, endothelial progenitor cells, bone marrow and peripheral blood mononuclear cells, and adipose stem cells, to improve DFU healing outcomes in preclinical animal models and human clinical trials. We also review novel technologies, such as iPSC-derived cell sources, CRISPR gene editing, and 3D human tissue models, to generate and modify stem cells that can give rise to multiple cell types needed for DFU healing and to streamline their preclinical testing. By further understanding how stem cells and other new technologies can best stimulate tissue regeneration, we will be able to overcome existing barriers to improve DFU therapies.

Abbreviations

ASCs	Adipose-derived stem cells
BM-MNCs	Bone marrow-derived mononuclear cells
BM-MSCs	Bone marrow-derived mesenchymal stem cells
DFU	Diabetic foot ulcers
ECM	Extracellular matrix
EPCs	Endothelial progenitor cells
HSCs	Hematopoetic stem cells
HSEs	Human skin equivalents
iPSCs	Induced pluripotent stem cells
PBMCs	Peripheral blood mononuclear cells
UCB	Umbilical cord blood

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Introduction

The cellular complexity of diabetic foot ulcers (DFUs) presents both opportunities and challenges for the development of new cell therapies designed to stimulate the healing of chronic wounds. Temporally and spatially coordinated interactions between multiple cell types and their products, such as extracellular matrix (ECM) proteins and soluble cytokines and chemokines, are critical for wound closure [1–4]. Cell therapy for DFUs must overcome the compromised cellular activity that limits activation of a regenerative stroma. Impaired mechanisms of wound repair in nonhealing DFUs are manifested by alterations in neovascularization, in deposition, organization, and remodeling of ECM, in growth factor-mediated cellular cross talk, and in impaired reepithelialization [5–13].

Stem cell therapy is emerging as a promising treatment modality aimed at improving processes underlying the pathophysiology of DFUs. Stem cells have been shown to home to wounds where they are mobilized to secrete chemokines and growth factors that promote angiogenesis and ECM remodeling, thus contributing to a local environment conducive to wound healing [14–17]. A key question facing the success of future cell therapies is whether the delivery of these cells to DFUs has the potential to activate the repair processes that are deficient in these nonhealing ulcers. In light of the multiple cellular defects that contribute to DFUs, it is essential to identify and study cell sources that can restore a range of normal cellular functions. It is likely that multiple cell types will need to be transplanted, and then persist and function at the wound site in order to

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jump-start and sustain wound healing in ways that advance the treatment of DFUs.

Stem cells used to promote healing in DFUs can be categorized into allogeneic and autologous cell sources, based on their relation to the donor from whom they are derived. Allogeneic stem cells are defined as cells that are derived from the same species as the recipient but are neither genetically matched nor immunologically compatible with the subject receiving them. Allogeneic cells persist for a limited amount of time during which they may recruit other cells that activate biological processes necessary for wound repair [18–20]. In contrast, autologous stem cells are cells collected from and then used in the same individual. Autologous cells have been shown to survive at the site of delivery to activate wound repair processes. These cell therapies are often evaluated in xenogeneic models, in which these human cells are tested for treatment in other species.

Stem Cells and DFU Therapy

Examples of stem cells used in cell therapies include bone marrow-derived mesenchymal stem cells (BM-MSCs), hematopoetic stem cells (HSCs), endothelial progenitor cells (EPCs), bone marrow-derived mononuclear cells (BM-MNCs) and peripheral blood mononuclear cells (PBMCs), endothelial progenitor cells (EPCs), and adipose-derived stem cells (ASCs). This chapter will summarize the progress in the use of these sources of stem cells to heal and repair DFUs (Fig. 13.1). We will review the animal studies in which xenogeneic, allogeneic, and autologous sources of stem cells have been tested and screened to enable future clinical trials (Table 13.1). We will also review human clinical trials in which allogeneic and autologous sources of stem cells have been tested for therapy (Table 13.2). We will discuss known therapeutic benefits of existing sources of adult stem cells and will discuss future directions for stem cell

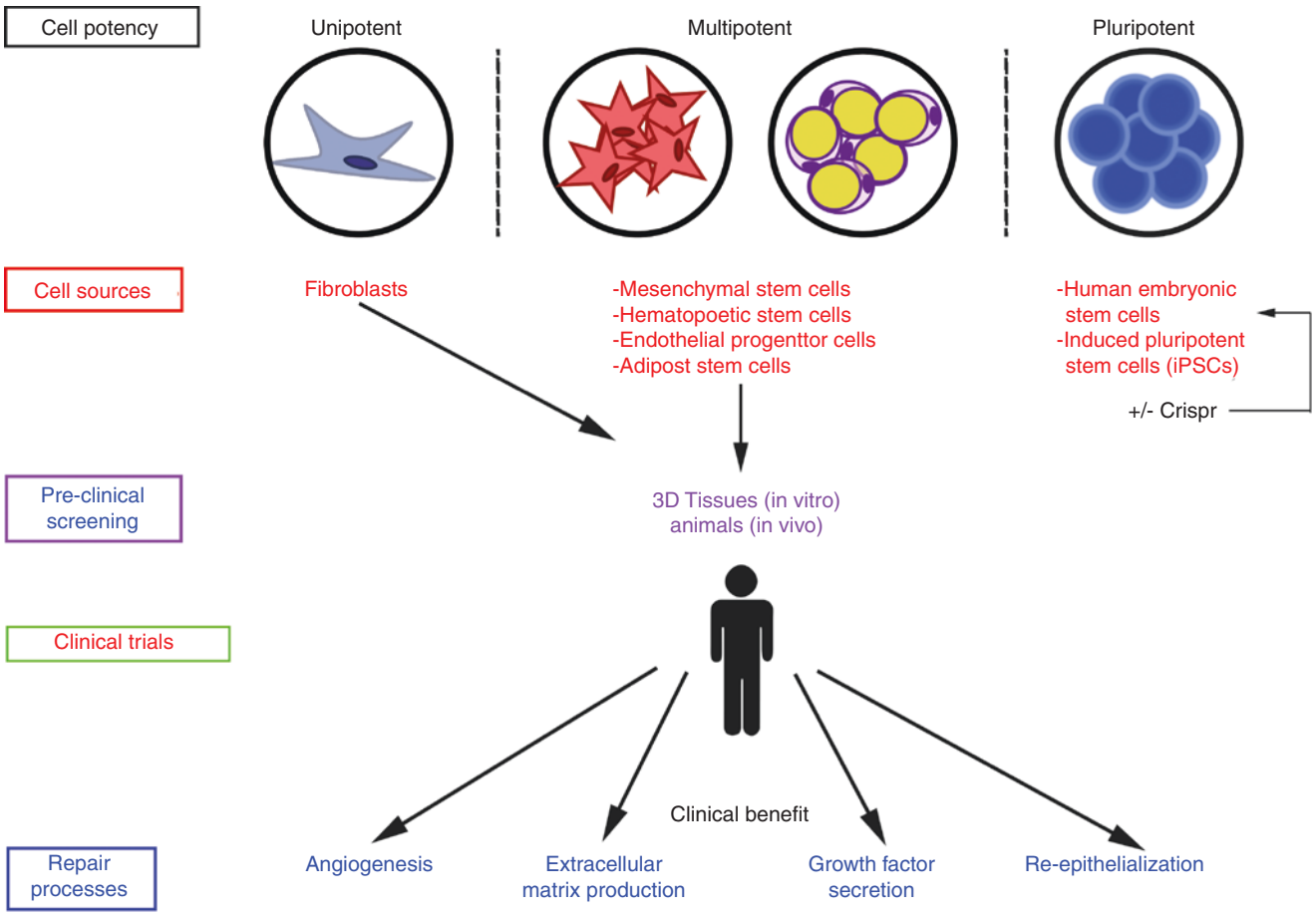

Fig. 13.1 Potency, source, and functions of stem cells used to treat DFUs. Multipotent and unipotent cells are derived from various tissues and classified based on their capacity to differentiate to functional cell types. Pluripotent stem cells can be differentiated into nearly all cell types and can be modified using CRISPR gene edited. These cell types can be screened and tested in 3D, in vitro human tissue models and subsequently in animal models before being tested in human clinical trials. This will determine the clinical benefit through wound repair processes such as angiogenesis, extracellular matrix production, growth factor secretion, and reepithelialization

Table 13.1 In vivo animal studies of diabetic foot ulcer therapies

Cell type	Animal model	Outcome	References
Bone marrow mesenchymal stem cells			
BM-MSCs	Rats/nondiabetic	Improved in healing compared to skin derived fibroblasts	McFarlin et al (2006) [29]
BM-MSCs	Mice/diabetic	Reepithelialization enhanced by application of BM-MSCs to injury site	Javazon et al. (2007) [32]
BM-MSCs	Mice/diabetic	Improved angiogenic response	Wu et al. (2007) [33]
BM-MSCs + chemokines	Mice/nondiabetic	BM-MSCs combined with chemokines more effective	Sasaki et al. (2008) [25]
BM-MSCs	Mice/nondiabetic	Chemokine release enhanced wound healing	Chen et al. (2008) [19]
BM-MSCs (Xenograft)	Rabbit/nondiabetic	Inhibited scar formation and increased tensile strength of full-thickness cutaneous wounds	Stoff et al. (2009) [30]
BM-MSCs (autograft, allograft, xenograft)	Pig/nondiabetic	Treatment with autologous, allogeneic or xenogeneic cells induced wound healing and regeneration of skin and hair follicles	Mansilla et al. (2010) [31]
BM-MSCs + EGF	Mice/diabetic	Increased blood flow with EGF compared to BM-MSCs alone (70%)	Amin et al. (2010) [34]
BM-MSCs	Mice/diabetic	Streptozotocin-induced Type I diabetic mouse treated with BM-MSCs resulted in an increase in tissue regeneration biomarkers and decrease in pro-inflammatory markers	Kuo et al. (2011) [36]
BM-MSCs	Rats/nondiabetic	3D collagen allograft with BM-MSCs upregulated MMP-9 promoting repair	Kim et al. (2011) [55]
BM-MSCs	Rats/diabetic	Increased VEGF and improved granulation tissue at the wound site	Wan et al. (2013) [35]
Endothelial progenitor cells			
EPCs	Mice/nondiabetic	Human isolated EPCs injected systemically into nude mice with ischemia selectively localized to areas of ischemia and induced new blood vessel growth	Park et al. (2004) [50]
EPCs	Mice/nondiabetic	EPC injection in mice dermal excisional wound model promoted wound closure	Suh et al. (2005) [20]
EPC delivery systems	Mice/nondiabetic	Bioactive material was most effective method of EPC delivery for improving vascularization in ischemic mouse hind limb	Silva et al. (2008) [53]
Fetal aorta-derived EPCs	Mice/diabetic	Wound healing and angiogenesis in streptozotocin-induced diabetic mice	Barcelos et al. (2009) [54]
Embryonic-EPCs CM	Mice/nondiabetic	Paracrine factors released from cultured media of human embryonic epithelial progenitor stimulated wound healing in nude mouse	Lee et al. (2011) [56]
EPCs	Mice/diabetic	Allogenic EPCs from normal diabetic mice accelerated wound closure compared to autologous EPCs	Marrotte et al. (2010) [51]
EPCs	Mice/diabetic	EPCs that were applied topically to wounds on the diabetic mice accelerated wound closure	Asai et al. (2013) [52]
EPCs	Mice and rabbits/ diabetic	Observed inconsistencies at the cellular level among diabetic humans, mice and rabbits	Tecilazich et al. (2013) [15]
Mononuclear stem cells			
PB-MNCs	Mice/diabetic	Human PB-MNCs decreased wound size in diabetic mice	Sivan-Loukianova et al. (2003) [67]
PBMCs + fibroblasts	Mice/diabetic	PBMCs mixed with fibroblasts accelerated wound healing in mice	Ueno et al. (2016) [68]
Adipose stem cells			
ASCs	Mice/diabetic	ASCs promoted neovessel formation and better tissue remodeling in treated mice compared with the control group.	Kim et al. (2011) [28]
ASCs	Mice/diabetic	ASCs overexpressing SDF-1 promoted the healing of wound in STZ-induced diabetic mice	Di Rocco et al. (2010) [80]
ASCs	Rat/diabetic	ASCs enhanced wound healing in STZ-induced diabetic rat model, but did not enhance angiogenesis	Maharlooei et al. (2011) [81]
ASCs	Rat/diabetic	ASCs improved vasculogenesis and accelerated wound closure in both normal and diabetic rat model	Nie et al. (2011) [82]
ASCs	Rat/diabetic	Autologous ASC transplantation enhanced skin graft survival in diabetic rats, and promoted angiogenesis through the secretion of growth factors such as VEGF	Zografou et al. (2013) [83]

(continued)

Table 13.1 (continued)

Cell type	Animal model	Outcome	References
ASCs	Mice/diabetic	ASCs harvested from nondiabetic mice had significantly improved wound healing outcomes when compared to autologous diabetic ASCs	Cianfarani et al. (2013) [84]
ASCs	Mice/nondiabetic	Single-layer sheets of ASCs sheet delivery system on wound	Lin et al. (2013) [85]
ASCs	Mince/nondiabetic	Multilayered ASCs sheets promoted a thicker epidermal surface in a mouse wound than single-layered ASC sheets	McLaughlin et al. (2013) [86]
ASCs	Mice/diabetic	Significantly advanced granulation tissue formation, capillary formation, and epithelialization at wound site treated with ASCs and matrix	Nambu et al. (2011) [10]
ASCs	Mice/nondiabetic	Cell sheet delivery limitations due to poor neovascularization	Cerqueira et al. (2013) [87]
ASC cell sheets	Mice/diabetic	ASC sheets combined with artificial skin accelerated wound healing in type 2 diabetic mice	Kato et al. (2015) [88]
ASCs	Mice/diabetic	Cell sheets that were combined with artificial skin accelerated wound healing and more rapid vascularization	Jiang et al. (2013) [89]

Table 13.2 Human/clinical trials of diabetic foot ulcer therapies

Bone marrow mesenchymal stem cells			
BM-MSCs	Topical fibrin spray system	Both diabetic and nondiabetic wounds healed	Falanga et al. (2007) [37]
BM-MSCs	Artificial collagen sponge	The improved wound healing in 18 of 20 patients	Yoshikawa et al. (2008) [38]
BM-MSCs	Standard wound dressing	Improvement in pain-free walking distance and reduction in DFU size	Dash et al. (2009) [39]
BM-MSCs vs. PBMCs	Injection	DFU healing with BM-MSCs more effective than BM-MNCs	Lu et al. (2011) [40]
U-MSCs	Endovascular infusion and injection	28 DFU patients showed significant ulcer healing from U-MSCs treatment following angioplasty	Qin et al. (2016) [41]
Endothelial progenitor cells			
EPCs	Injection and topical application	Complete wound healing achieved 4 ulcer patients, 1 of which due to diabetes	Badiava et al. (2007) [57]
G-CSF stimulated peripheral blood CD34+ progenitor cells	Subcutaneous injection	Complete DFU closure in all 5 patients	Tanaka et al. (2014) [43]
Mononuclear stem cells			
BM-MNCs	Injection	Dermal rebuilding and closure of nonhealing chronic wounds were achieved in all 3 patients	Badiavas and Falanga (2003) [18]
BM-MNCs	Subcutaneous injection	Reduction of wound size and increased vascularization for the 1 patient	Humpert et al. (2005) [65]
BM-MNCs	Injection	Complete wound healing achieved in the 1 patient	Kirana et al. (2007) [66]
BM-MNCs	Intra-arterial implantation	Wound healing and neovascularization seen in 20 diabetic patients	Ruiz-Salmeron et al. (2011) [63]
BM-MNCs + platelets + fibrin glue + collagen matrix	Injection and topical application	Complete wound healing in 3 cases and significant improvement in the remaining 5 cases	Ravari et al. (2011) [64]
BM-MNCs alone vs. combined with BM-MSCs	Intramuscular injection or intra-arterial	18 out of 22 patients showed significant improvement	Kirana et al. (2012) [71]
PB-MNCs	Intramuscular injection	Increased blood flow and improved angiogenesis in patients treated with PBMCs	Ozturk et al. (2012) [69]
BM-MNCs vs. PB-MNCs	Injection	BM-MNC and PBMC treatments in patients with DFUs and critical limb ischemia (CLI) have no significant difference in therapeutic outcome	Dubsky et al. (2013) [70]
Adipose stem cells			
ASCs	Intramuscular injection	DFU patients with critical limb ischemia showed improved ulcer healing rates	Lee et al. (2012) [90]
ASCs	Injection	All 3 patients with DFU treated showed improved clinical outcomes	Marino et al. (2013) [91]
ASCs + e-PRP	Injection	ASC in combination of e-PRP increased ulcer healing rater in 3 of 3 diabetic patients	Raposio et al. (2016) [92]

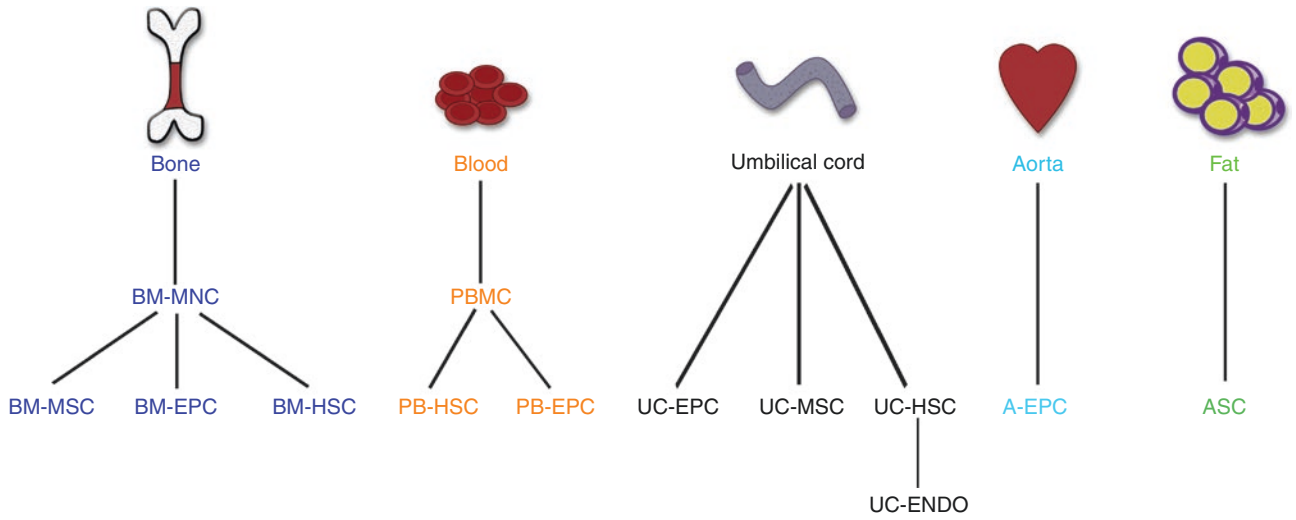

Fig. 13.2 Sources of adult stem cells used for treatment of Diabetic Foot Ulcers. Adult stem cells can be obtained from a variety of sources including bone marrow, umbilical cord, peripheral blood, fetal aorta, and adipose tissue. These stem cells have been tested in animal and human studies and shown to manifest cellular functions that improve the repair of diabetic foot ulcers. *BM-MNC* bone marrow-derived mononuclear cell, *BM-MSC* bone marrow-derived mesenchymal stem cell, *BM-EPC* bone marrow-derived endothelial progenitor cell, *BM-HSC* bone marrow-derived hematopoetic stem cell, *PBMC* peripheral blood mononuclear cell, *PB-HSC* peripheral blood hematopoetic stem cell, *PB-EPC* peripheral blood endothelial progenitor cell, *UC-EPC* umbilical cord endothelial progenitor cell, *UC-MSC* umbilical cord mesenchymal stem cell, *UC-HSC* umbilical cord hematopoetic stem cell, *UC-ENDO* umbilical cord endothelial cell, *A-EPC* aorta endothelial progenitor cell, *ASC* adipose stem cell

treatment of DFUs using novel sources of stem cells, such as induced pluripotent stem cells (iPSCs). We will also examine the genetic modification of these cells through gene editing tools (CRISPR/Cas9) to improve treatments for DFUs.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are found in many tissue types, including bone marrow, umbilical cord blood, adipose tissue, dermis of skin, and amniotic membrane [21, 22] (Fig. 13.2). Bone marrow-derived mesenchymal stem cells (BM-MSCs), also known as marrow stromal cells, are the most common source of MSCs for clinical use in DFU treatment [23]. These clinical applications are based on the well-characterized plasticity of MSCs, as seen by their capacity to differentiate into mesenchymal cells such as osteoblasts, adipocytes, and chondroblasts in vitro [24]. Additionally, Sasaki et al. (2008) demonstrated that mouse BM-MSCs can differentiate into non-mesenchymal lineages, including keratinocytes, endothelial cells, and pericytes [25]. This multipotent differentiation potential, their ability to be grown and expanded efficiently in tissue culture, and their weak immunogenicity and strong immunoregulatory effects make them a useful source of cells that can stimulate human tissue regeneration [26].

The utilization of the cellular plasticity of MSCs for DFU therapy has been demonstrated in preclinical animal and clinical human studies. These studies showed accelerated wound closure through MSC modulation of the inflammatory environment, recruitment of inflammatory cells, promotion of neovascularization, and regeneration of appendages [27]. BM-MSCs appear to be critical for wound repair as seen by elevated production of cytokines known to enhance wound healing. These include epidermal growth factor (EGF), keratinocyte growth factor (KGF), insulin like growth factor 1 (IGF-1), vascular endothelial growth factor A (VEGF-A), erythropoietin (EPO), stromal cell-derived factor 1(SDF-1), macrophage inflammatory protein 1 alpha and beta (MIP-1 α/β), and transforming growth factor beta (TGF-β). The release of these cytokines by BM-MSCs has been shown to recruit additional MSCs to the wound site, leading to their differentiation into a spectrum of healing-competent cell types [19].

The potential for using MSCs for treatment of DFUs was first established using animal models of normal wound repair [28–31]. The efficacy of BM-MSCs in healing acute wounds was shown in nondiabetic rats, where their topical application significantly improved repair when compared to application of fibroblasts. The BM-MSCs increased collagen production, wound strength, and growth factor secretion [29]. In another study, BM-MSCs, applied as a collagen allograft to full-thickness wounds on the dorsum of rats, stimulated production of the ECM-degrading enzyme matrix metalloproteinase-9 (MMP-9), which mobilized the angiogenic growth factor VEGF to accelerate wound healing [28]. Intradermal injection of human BM-MSCs improved the repair of full-thickness incisional wounds in rabbits.

The study also showed that these cells inhibited scar formation while increasing tensile strength in the acute, nondiabetic wounds [30]. Similarly, skin regeneration of porcine burn wounds was improved using acellular dermal matrices when combined with autologous, allogeneic, and xenogeneic BM-MSCs, which resulted in skin and appendage regeneration with little scarring [31].

These studies in nondiabetic animal models paved the way for preclinical studies in diabetic animals which demonstrated that BM-MSCs can enhance wound repair through improved reepithelialization and granulation tissue formation, stromal activation, and increased angiogenesis [32, 33]. The role of BM-MSCs in the stimulation of blood vessel ingrowth was seen in a diabetic mouse model of hind-limb ischemia, where a dramatic increase in blood flow and increased vessel density was seen in the presence of BM-MSCs, which was further augmented in the presence of epidermal growth factor (EGF) [34]. Similar results were found following the subcutaneous injection of BM-MSCs in a diabetic rat, which led to enhanced wound healing by promoting angiogenesis, cellular proliferation, and augmented granulation tissue thickness [35]. Additionally, when BM-MSCs were topically applied to wounds in streptozotocin-induced Type I diabetic mice, they significantly enhanced wound healing by increasing angiogenesis (increased VEGF secretion), decreasing inflammation (decreased leukocyte recruitment), and increasing tissue remodeling [36].

These diabetic animal studies provided proof of concept for the application of BM-MSCs in human clinical trials (Table 13.2). Human BM-MSCs have been shown to effectively heal DFUs when they were delivered using a spray delivery system consisting of fibrinogen and thrombin [37]. When BM-MSCs were incorporated into an artificial dermis consisting of a collagen sponge and implanted subcutaneously, the DFU patients demonstrated regeneration of subcutaneous tissues and a robust vascular response [38]. Wound dressings delivering BM-MSCs were found to effectively treat DFU patients, resulting in reduced pain and ulcer size following dermal regeneration [39]. Improved angiogenic responses induced by BM-MSCs were also seen when these cells were injected into Type II diabetic patients with lower limb [40]. Additionally, umbilical cord mesenchymal stem cells (UC-MSCs) were found to reduce lower limb amputation rates and increase vessel count, suggesting that UC-MSCs improved angiogenic responses [41].

Hematopoetic Stem Cells

Hematopoetic stem cells (HSCs) are CD45-positive and CD34-positive progenitor cells that give rise to all differentiated blood cell types. HSCs are the most abundant cell type in the bone marrow, but can also be isolated from peripheral or umbilical cord blood (UCB) (Fig. 13.2). HSCs have been shown to enhance DFU healing in both the inflammatory and proliferative phases of diabetic wounds [16, 42, 43]. It was shown that UCB-derived HSCs had the capacity to differentiate into endothelial cells that when combined with HSCs could heal wounds in diabetic mice when applied in a topical gel [44]. Similar findings were shown in human studies when UCB-derived HSCs were combined with UCB-derived MSCs to augment granulation tissue production and improve DFU healing [45].

Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) are recruited from the bone marrow (BM-EPCs) into the peripheral circulation in response to specific stimuli, where they acquire the capacity to stimulate angiogenesis and wound repair [15, 46, 47] (Fig. 13.2). These cells display surface markers that are typical of both endothelial and hematopoietic cells and for vascular structures important for vasculogenesis and angiogenesis [48]. BM-EPCs are found in decreased numbers in diabetic patients, thus suggesting a link between reduced BM-EPCs and DFUs [16]. Tecilazich et al. (2013) also found that numbers of EPCs were reduced in patients at risk for developing DFU [15].

A recent study in diabetic mice demonstrated decreased numbers of EPCs not only in the peripheral blood but also in the wound bed. The number of EPCs was increased after blocking pro-inflammatory receptor CXCR4 with its antagonist. Furthermore, the treated mice had increased angiogenesis and cell proliferation, with subsequent rapid wound closure, suggesting an enhancement of DFU healing due to increased numbers of EPCs [49]. Human BM-EPCs have been injected into the circulation of nude mice where they have been found to localize to areas of tissue ischemia and induce new blood vessel growth. The authors suggested that human BM-EPC transplantation could have a potential role in the regeneration of ischemic tissue in humans [50]. In Type II diabetic mice, BM-EPCs collected from normal mice accelerated wound closure compared to BM-EPCs collected from diabetic mice [51]. BM-EPCs that were applied topically to full-thickness wounds on the dorsum of diabetic mice were found to accelerate wound closure and increase vascularity [52]. In ischemic mouse hind limbs, BM-EPCs, delivered in a bioactive material, accelerated wound closure [53].

EPCs derived from other sources have also been shown to improve wound healing. Human fetal aorta-derived EPCs have been found to stimulate wound healing and promote angiogenesis in streptozotocin-induced diabetic nude mice [54], while human cord blood-derived EPCs activated keratinocyte and fibroblast proliferation to stimulate

wound closure [55]. Injection of peripheral blood-derived EPCs into mice accelerated wound reepithelization and improved macrophage responses when compared to injection with mature endothelial cells [20]. These therapeutic effects were also seen using endothelial cell conditioned media, suggesting that the delivery of paracrine growth factors may be a viable therapeutic option instead of cells. It was found that the secreted growth factors released into cultured media by EPCs derived from human embryonic stem cells were able to successfully close wounds in a nude mouse model [56].

Relatively few human clinical studies have been performed to assess potential benefits of EPCs in the treatment of DFUs. Badiavas et al. [57] showed increased blood vessel growth and wound closure when BM-EPCs were topically applied to or injected in DFUs. DFUs have shown improved wound closure and vascular perfusion when peripheral blood-derived EPCs were stimulated with the cytokine granulocyte colony-stimulating factor (G-CSF) in a prospective clinical trial. This trial showed that combined therapies of CD34-positive stem cells and cytokines could offer a safe and efficacious DFU treatment [43].

Mononuclear Stem Cells

Mesenchymal stem cells, hematopoetic stem cells, and endothelial progenitor cells are all found in the mononuclear cell fraction derived from bone marrow or peripheral blood [58–61] (Fig. 13.2). This heterogeneous population of mononuclear cells has been used collectively in cell therapy applications for treating diabetic ulcers [62]. Autologous bone marrow-derived mononuclear cells (BM-MNCs) were found to stimulate wound vascularization following their intra-arterial delivery in DFU patients with peripheral artery disease [63]. An alternative approach was used to improve healing of DFUs that were refractory to other treatments by delivering BM-MNCs in a collagen-fibrin gel [64]. Multiple case reports have demonstrated a similar efficacy of BM-MNCs to improving both DFU repair and angiogenic responses in diabetic patients [65, 66].

As an alternative to BM-MSCs, peripheral blood mononuclear cells (PBMCs) have been found to improve wound healing. Human-derived PBMCs alone [67] and PBMCs mixed with fibroblasts [68] have both been found to accelerate epidermal wound healing in mice. Importantly, patients with Type II diabetes with DFUs and limb ischemia showed increased blood flow and improved angiogenesis when treated with PBMCs [69].

Since both PBMCs and BM-MNCs have shown efficacy for DFU therapy, it is important to determine if there are relative benefits for each. Dubsky et al. (2013) compared BM-MNC and PBMC treatments in patients with DFUs and critical limb ischemia (CLI) and reported no significant difference in therapeutic outcome [70], suggesting that there is no difference in their healing potential. Additionally, Lu et al. (2011) explored differences in safety and efficacy between BM-MNCs and BM-MSCs. In this study, patients with Type II diabetes exhibiting DFUs and CLI and treated with BM-MSCs were found to have greater healing than with BM-MNCs [40]. In contrast, when treatments with BM-MNCs or BM-MSCs were compared using two different delivery methods (intramuscular injection vs. intra-arterial infusion), it was found that both types of cells showed similar healing responses [71].

Adipose Tissue-Derived Stromal Cells

Adipose tissue-derived stromal cells (ASCs) are mesenchymal cells found in adipose tissues that are able to differentiate into a spectrum of cell types that may be important for wound healing [72] (Fig. 13.2). When ASCs were transplanted into nondiabetic animal wounds they were shown to promote reepithelialization and angiogenesis [73–78]. Responses to human-derived ASCs have been studied in diabetic mouse and rat models, where stimulation of angiogenesis and improved tissue remodeling has been seen [79]. Genetically modified ASCs designed to overexpress the chemokine stromal-derived factor-1 (SDF-1) promoted healing in STZ-induced diabetic mice. Di Rocco et al. and Maharlooei et al. [80, 81] used ASCs to treat STZ-induced diabetic rats and found accelerated wound healing that was linked to a decrease in the density of fibroblasts, suggesting that improved diabetic wound healing was likely to be controlled by mechanisms other than accumulation of collagen produced by fibroblasts. Nie et al. [82] demonstrated that autologous ASC treatment in diabetic rats closed wounds and resulted in the elevated expression of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and fibroblast growth factor 2 (FGF2). The utility of ASCs was further supported when autologous ASCs combined with full-thickness skin grafts were used in a diabetic rat model. ASCs enhanced skin graft survival and resulted in greater collagen density, increased levels of VEGF, and improved angiogenesis [83]. Together these studies support previous findings that ASCs promote new vessel formation and granulation tissue deposition.

When the efficacy of ASCs from diabetic and nondiabetic mice were compared in wound healing in a diabetic mouse, it was found that ASCs harvested from nondiabetic mice had significantly improved wound healing outcomes when compared to autologous, diabetic ASCs. ASCs from nondiabetic mice stimulated significantly greater amounts of granulation tissue, collagen deposition, and vessel density in diabetic wounds [84].

Novel approaches have been used for the delivery of ASCs to wound sites. Lin et al. (2013) formed single-layer sheets of ASCs by culturing ASCs in a monolayer on a temperature sensitive *N*-isopropylacrylamide (PIPAAm). This allows for formation of ASCs sheets which can be easily detached from the cell culture surface and layered on top of each other. These multilayered cell sheets generated greater collagen density in the wounds of athymic nude mice than single-layered sheets [85]. Similarly, McLaughlin et al. (2013) found that such multilayered ASCs sheets promoted a thicker epidermal surface in a mouse wound than single-layered ASC sheets and ASCs generated on standard cell culture surfaces [86]. Other studies have examined seeding ASCs in an artificial dermal substitute and placing it directly on a wound created on diabetic mice. The treatment significantly enhanced granulation tissue formation, capillary formation, and epithelialization [10]. However, skin substitutes for stem cell delivery have been limited by the poor vascularization of these scaffolds [87]. To overcome this obstacle of cell delivery, Kato et al. (2015) treated skin defects in Type II diabetic rats using allogeneic ASCs that were incorporated into cell sheets that were combined with artificial skin, resulting in accelerated wound healing and more rapid vascularization than with either treatment alone [88]. Similarly, Jiang et al. (2013), using a diabetic, porcine wound model, found that the combination of collagen scaffolds and autologous ASC sheets resulted in higher vascularization and expression of VEGF when compared to ASC sheets alone or topically applied ASCs [89]. Thus, the combination of three-dimensional scaffolds with ASC sheets provides new a delivery modality to accelerate wound closure and enhance angiogenesis, cell migration, and proliferation.

DFU patients with critical limb ischemia and injected intramuscularly with cells differentiated from ASCs showed clinical improvement as evidenced by improved claudication, healed amputation sites, lengthened walking distances, increased ulcer healing rates, and formation of numerous vascular collateral networks [90]. Similarly, Marino et al. (2013) showed improved healing in patients with diabetic ulcers and peripheral arterial disease after intradermal injection of ASCs harvested from nondiabetic patient donors [91]. The treatment of nonhealing chronic ulcers, including DFUs, was also enhanced when ASCs were used with or without enhanced Platelet-Rich-Plasma (e-PRP) [92].

In summary, bone marrow-derived mesenchymal stem cells, hematopoetic stem cells, endothelial progenitor cells, bone marrow-derived and peripheral blood mononuclear cells, and adipose tissue-derived stem cells have all been shown to improve wound healing in preclinical animal models and human clinical trials. While great strides have been made in using stem cell therapy for treating diabetic foot ulcers with these stem cell types, the therapeutic mode of action of these cells remains to be elucidated. Importantly, a limitation of these sources is that they do not supply an unlimited quantity of autologous cells for therapy.

Future Directions and New Technologies

A new replenishing source of multiple cell types important for wound healing is induced pluripotent stem cells (iPSCs). In addition, gene editing strategies, such as CRISPR/Cas9, are emerging as promising technologies to modify specific genes, thus altering the production of proteins that may improve wound healing in patients with DFUs. We will review the principles of these two novel technologies and will discuss their potential impact on DFU therapy.

Induced Pluripotent Stem Cells

James Thomson's landmark study in 1998 discovered a method for isolating human embryonic stem cells (hESCs) (Thomson et al. 1998). These pluripotent stem cells were shown to give rise to cells from all three embryonic germ layers and possibly primordial germ cells (PGCs) [93]. hESCs have the potential to be used as a source of cells for regenerative medicine, however, due to ethical and legal issues regarding research using human embryos, progress on the clinical application of these cells has been limited. An alternative source of pluripotent stem cells was discovered when [94] showed that mouse embryonic fibroblasts (MEFs) could be reprogrammed to a state that was similar to mouse embryonic stem cells by using only four transcription factors (OCT-4, KLF4, SOX2, c-MYC (OKSM)) [94]. In 2007, this approach was successfully implemented to reprogram human fibroblasts to a pluripotent state. These cells, known as "induced pluripotent stem cells" (iPSCs), show many of the same self-renewal and differentiation capabilities as hESCs. The same reprogramming approach has now been applied to a wide variety of somatic cells sources [95–102].

The ability to reprogram many types of somatic cells into iPSCs shows promise for diabetic patient-specific cell derivation. iPSCs have been generated from skin fibroblasts of patients with maturity onset diabetes of the young (MODY) [103, 104], patients with Type I and Type II diabetes [105–107], Juvenile-onset Type I diabetes [108], and directly from DFU-derived fibroblasts in patients with Type II diabetes [109]. Gerami-Naini et al. [109] successfully reprogrammed primary fibroblast cell lines derived from DFUs to iPSCs and compared them to iPSCs derived from non-ulcerated foot skin from diabetic patients and from healthy foot skin from nondiabetic patients. These studies have established that primary, DFU-derived fibroblasts can be reprogrammed with efficiencies similar to nondiabetic control fibroblasts, thus holding promise for future diabetic patient-specific regenerative therapy of DFUs.

If iPSCs are going to serve as an improved source of cells for both autologous and allogeneic cell therapies, it will be necessary to differentiate them into the multiple cell types needed for the treatment of DFUs. This is feasible goal, as

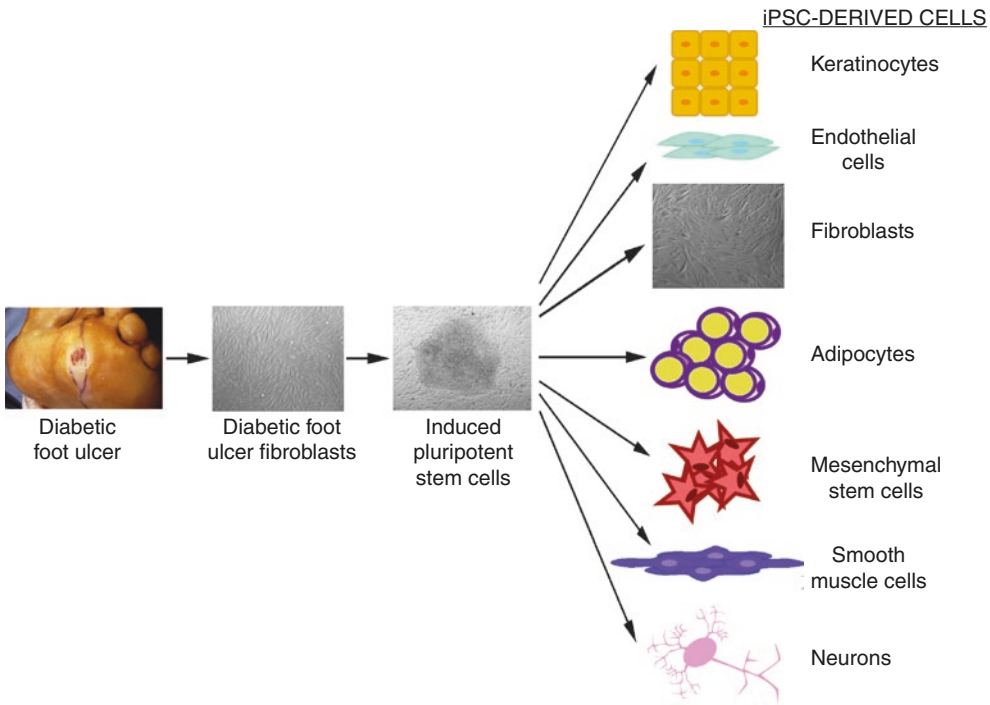

Fig. 13.3 Cells differentiated from iPSCs for use as a cell source for DFU treatment. iPSCs reprogrammed from many cell types and directly from DFUs can be differentiated into multiple cell types needed for DFU treatment, including keratinocytes, endothelial cells, adipocytes, mesenchymal stem cells, smooth muscle cells, and neurons

pluripotent stem cells have previously been differentiated into endothelial cells [110–113], smooth muscle cells [111, 114], adipocytes [115, 116], fibroblasts [117–119], keratinocytes [120–125], motor and sensory neurons [126–129], and mesenchymal stem cells [117, 130–134]. All of these are relevant for improving wound healing in patients with diabetic foot ulcers. However to date, iPSC-derived cells that were initially reprogrammed from DFU-derived fibroblasts have only been used to generate cells in a fibroblast lineage [109] (Fig. 13.3).

Improvements in reprogramming methods for iPSC generation will be required to ensure the safe and effective use of iPSCs for DFU treatment in clinical trials. One barrier to the safe clinical translation of iPSCs [135] is the use of lentiviral- and retroviral-based vectors to express the OKSM reprogramming factors in a somatic cell of interest. These systems are both accompanied by transgene integration into the genome, leading to an increased risk of acquisition of harmful changes in gene expression. To address this problem, non-integrative viral approaches, such as adenovirus and Sendai virus, have been developed. However, the efficiency of iPSCs with adenovirus generation is much lower compared to lentivirus and retrovirus [136]. During the last decade, several nonviral reprogramming methods have also been established. These include delivering a plasmid vector [137], using episomal plasmids as "minicircle vectors" [97, 138], expressing OKSM mRNA [139], directly delivering OKSM proteins [140] or inducing expression of OKSM factors with microRNA [141] or with small molecules [142–144]. When these varied reprogramming methods have been compared, Sendai virus has been shown to be the most efficient means of reprogramming somatic cells to a pluripotent state without integration [145].

The risk of tumor formation from iPSC-derived cells is another challenge associated with their safe use for DFU treatment [146, 147]. This risk arises because formation of teratomas upon implantation into mice is the sine qua non for establishing the pluripotency of fully reprogrammed iPSCs [148, 149]. Thus, reliable in vitro differentiation protocols must be coupled with screening assays that will ensure the absence of residual pluripotent cells before use in DFU therapy [150]. Virus-free, transgene-free methods of reprogramming such as miRNA, episomal plasmid, non-integrating Sendai virus, and recombinant protein methods will reduce the risk of tumorigenicity that can result from viral-mediated, insertional mutagenesis. In addition, it is likely that iPSC-derived cells will be extensively manipulated ex vivo by the time that are ready for clinical use, which is known to lead to chromosomal aberrations and altered cell phenotypes [151, 152]. In light of this, cell therapies for DFUs will require extensive preclinical testing to ensure that cells differentiated from iPSCs do not contain genetic alterations, or harbor residual virus or pluripotent cells.

The efficacy and safety of several iPSC-derived cells are currently being tested in a small number of clinical trials. A recently initiated clinical trial is testing allogeneic iPSC-derived mesenchymoangioblasts as a treatment for acute graft versus host disease (AGVH) (clinicaltrials.gov ID NCT02923375). There are several ongoing clinical trials using allogeneic, iPSC- and hESC-derived retinal pigment epithelial cells as a treatment for wet age-related macular degeneration (AMD), dry AMD, and Stargardt's macular dystrophy

(clinicaltrials.gov ID NCT02464956, NCT01691261, NCT 01344993, NCT02463344, NCT02286089, NCT02755428, NCT01345006, NCT01469832, NCT02941991, NCT0244 5612, NCT02749734). A clinical trial designed to treat Type I diabetes is currently testing a product comprised of hESC-derived pancreatic endodermal cells and an immune-protecting encapsulation medical device (clinicaltrials.gov ID NCT02239354). hESC-derived oligodendrocyte precursor cells are also being evaluated to treat spinal cord injury (clinicaltrials.gov ID NCT01217008 and NCT02302157).

Gene Editing of DFU-Derived Cells for Stem Cell Therapy

Genome editing technologies, such as zinc finger nucleases [153], TALENs [154], and CRISPR/Cas9 [155, 156], can now be used to modify specific genes in primary DFU-derived cells or iPSCs to improve their wound healing potential. In recent years, genome editing has advanced dramatically with the discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), an important bacterial immune defense system [157–159] that has been harnessed to specifically target and modify genes with high efficiency. CRISPR technology relies on CRISPR-associated protein 9 (Cas9), which is an RNA-guided DNA endonuclease [89, 160], and a "guide RNA" (gRNA) consisting of CRISPR RNA (crRNA) fused to transactivating RNA. A crRNA is necessary for the precise targeting of the CRISPR/Cas9 complex to a specific sequence in the genome. Gene editing occurs as transactivating RNA binds Cas9 protein [155, 156, 161], making it possible for Cas9 to introduce a double strand break, disrupting the expression of the gene of interest. Alternatively, CRISPR/Cas9 can also correct a specific mutation, by inserting a sequence provided on the donor template.

At this point, a relatively small number of mutations associated with poor wound healing in diabetic patients have been identified, thus limiting the number of genes that could be targeted by gene editing. While the role of genetic mutations in the pathogenesis of DFUs remains poorly understood, numerous genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with Type I and Type II diabetes [162–165], that may prove to be useful gene editing targets in the future. For example, SNPs in IL-6, TNF-α, and SDF-1, which are genes known to be associated with DFUs [166], may be useful genomic targets that could be CRISPR modified in order to improve DFU healing. The potential use of CRISPR/Cas9 to treat DFUs will also be complicated by the multifactorial nature of chronic wound healing, which will likely necessitate modification of multiple genes in individual cells. Currently, up to nine different genomic loci have been simultaneously targeted in individual cells using CRISPR/Cas9 [155, 167–174]. While modifying multiple gene targets in the same cell may be feasible, the number of targets sufficient to improve wound healing in diabetic patients remains to be determined. Clinical applications of CRISPR/Cas9 technology will also require consideration of possible off-target effects, which can lead to unintended changes in expression of genes, requiring further optimization and refinement of Cas9 delivery [175–180]. Currently, CRISPR/Cas9 technology clinical trials have been approved only in China and for the treatment of cancer (ClinicalTrials.gov ID NCT02793856, NCT02867345, NCT02863913, NCT02867332).

As an alternative approach, the Cas9 protein can be repurposed to regulate transcription of genes that are dysregulated in the diabetic patients. For example, Cas9 protein with mutations in the nuclease domain cannot introduce double strand breaks, but can still be targeted to a specific locus using gRNA [181, 182]. This mutated Cas9 can be fused to enzymes which will then epigenetically regulate gene expression at that locus. Alternatively, a reversible CRISPR interference technique has been employed by targeting mutated Cas9 to the transcriptional start site, thus blocking gene transcription [183]. As genetic mutations may not be sufficient targets for editing in DFUs, it is likely that transcriptional modification using CRISPR/Cas9 will be useful in the future.

In summary, CRISPR/Cas9 technology holds future promise to modify specific diabetic gene signatures either by correcting disease-causing mutations or through epigenetic modification of expression of diabetes-associated genes that may lead to an improved cellular wound healing phenotype. Additionally, regulation of diabetes-induced gene expression may be achieved without CRISPR editing, simply by reprogramming primary DFU-derived cells to iPSCs [184], as it is known that cells undergo extensive transcriptional and epigenetic remodeling during reprogramming [185]. In conclusion, iPSC technology combined with CRISPR/Cas9 genome engineering will be a powerful approach to obtain cells with improved wound repair features that can be valuable for novel cell therapies needed to improve diabetic wound healing.

3D Tissue Models for Preclinical Drug Testing of DFU Therapies

The spatially and temporally controlled events that occur during tissue morphogenesis need to be studied in biological systems in which a high degree of tissue complexity can be achieved to recreate an in vivo-like tissue microenvironment. Biologically meaningful signaling pathways, including those that direct the proliferation and differentiation of epidermal

stem cells, function optimally when cells are spatially organized in 3D tissues rather than in rudimentary 2D, monolayer culture systems. In this light, the development and application of 3D tissue models that mimic healthy human skin and chronic wounds will play an important role in moving discovery of new treatments for DFU into a preclinical screening paradigm. Using bioengineered 3D tissues will therefore provide experimental systems that are characterized by growth factor-directed cell-cell cross talk and the presence of ECM-mediated cues that can recreate the complexity of conditions like DFUs. Existing preclinical therapeutic testing involves comparing the effects of stem cells on wound healing in both normal and diabetic animals. However, it is now clear that safety and efficacy testing of stem cell therapies for DFU treatment can be streamlined using 3D tissues.

One example of an exciting model system is human skin equivalents (HSEs) (Fig. 13.4). These are tissues fabricated by assembling a layer of primary fibroblasts in a collagen gel and placing atop a layer of keratinocytes grown at an air-liquid interface. Functional fibroblasts in the stromal layer consisting of Type I Collagen support the proliferation and differentiation of primary keratinocytes to form fully differentiated stratified epithelium [186, 187]. HSEs have been used to successfully model behaviors of normal skin and disease-specific tissues [188]. It has recently been shown that primary, DFU-derived fibroblasts can be incorporated into tissues models that mimic DFUs [189]. Maione et al. (2015) compared the phenotype of 3D tissues harboring either cells from the foot of healthy, diabetic, or diabetic foot ulcer patients and used them to compare differences in the phenotype of fibroblasts derived from these sources. The proliferation of surface keratinocytes was found to be elevated in the presence of DFU fibroblasts when compared to those from healthy donors, which simulates finding from DFUs in patients [189].

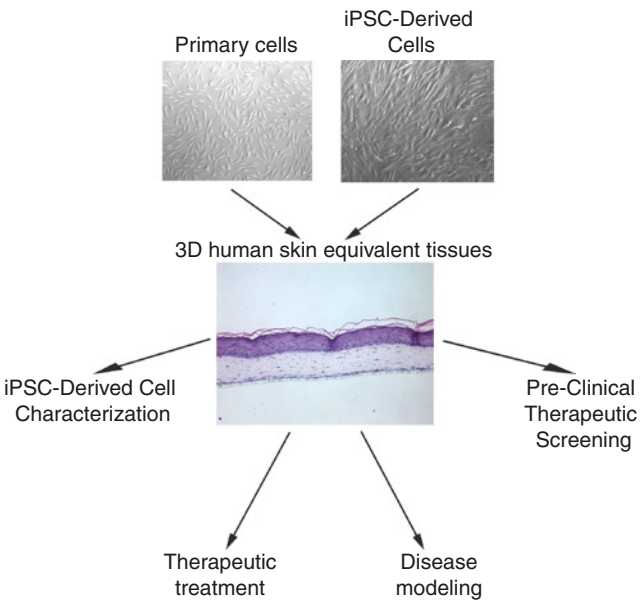

Fig. 13.4 Skin equivalent tissue model for testing cells for DFU therapy. Cells differentiated from primary and iPSC-derived cells can be used to construct 3D HSE tissues with many features of human skin. These tissues can incorporate patient-derived cells, as well as adult or iPSC-derived stem cells and for use in preclinical drug testing, disease modeling, and characterization of cell functions

In addition, a 3D model of acute wound healing using HSEs has been developed by creating a full-thickness wound in HSEs and monitoring reepithelialization of surface keratinocytes from the wound edge towards the wound center (Fig. 13.5) [186]. Maione et al. (2015) demonstrated that in this 3D wound healing model, DFU-derived fibroblasts showed delayed wound reepithelialization [189]. This wound healing model using diabetic patient-specific HSEs can now be used to effectively test the efficacy and safety of agents designed to accelerate wound repair before clinical trials. Additionally, HSEs can be integrated into 3D microfluidic devices in which multiple tissue types are grown, known as organs-on-chips, that can better mimic interactions between different tissue types to serve as a more predictive model for preclinical testing of cell therapeutics for DFUs [190, 191].

While these existing 3D tissue models of skin and other stratified epithelial tissues provide many benefits for mimicking human disease states, it would be advantageous to develop additional complexity in these tissues. Such complexity could be accomplished by incorporating additional cell types critical to DFU healing, such as macrophages and endothelial cells. A future step in the development of 3D skin-like tissues will be the ability to generate tissues that are personalized tissue "surrogates" of their in vivo counterparts. This will require the development of primary, patient-derived cell lines in which it will first need to be determined, in 2D culture during cell expansion, if the "identity" of these primary cells will be retained when incorporated into 3D tissues. Beyond this, 3D tissue models enable development of well-characterized benchmarks to confirm that cells differentiated from iPSCs have acquired cell phenotypes and functions that will best mimic features of skin. This can be accomplished by developing reproducible techniques for the derivation and characterization of iPSC-derived fibroblasts and other cell types that can assemble endogenous 3D ECM or provide soluble factors essential for optimal skin fabrication.

It has been shown that the tissue microenvironment plays a critical role in the epigenetic regulation of gene expression in 3D tissues [192]. This tells us that the impact of epigenetic alterations of gene expression on disease pathways is best studied in a complex 3D tissue context. It is clear that "surrogate" 3D tissues will elucidate mechanisms through which epigenetic control of gene expression and the 3D microenvironment drive the development of DFUs. This will enable the use of epigenetic targets in diabetic cells to be used as readouts of improved DFU wound healing response.

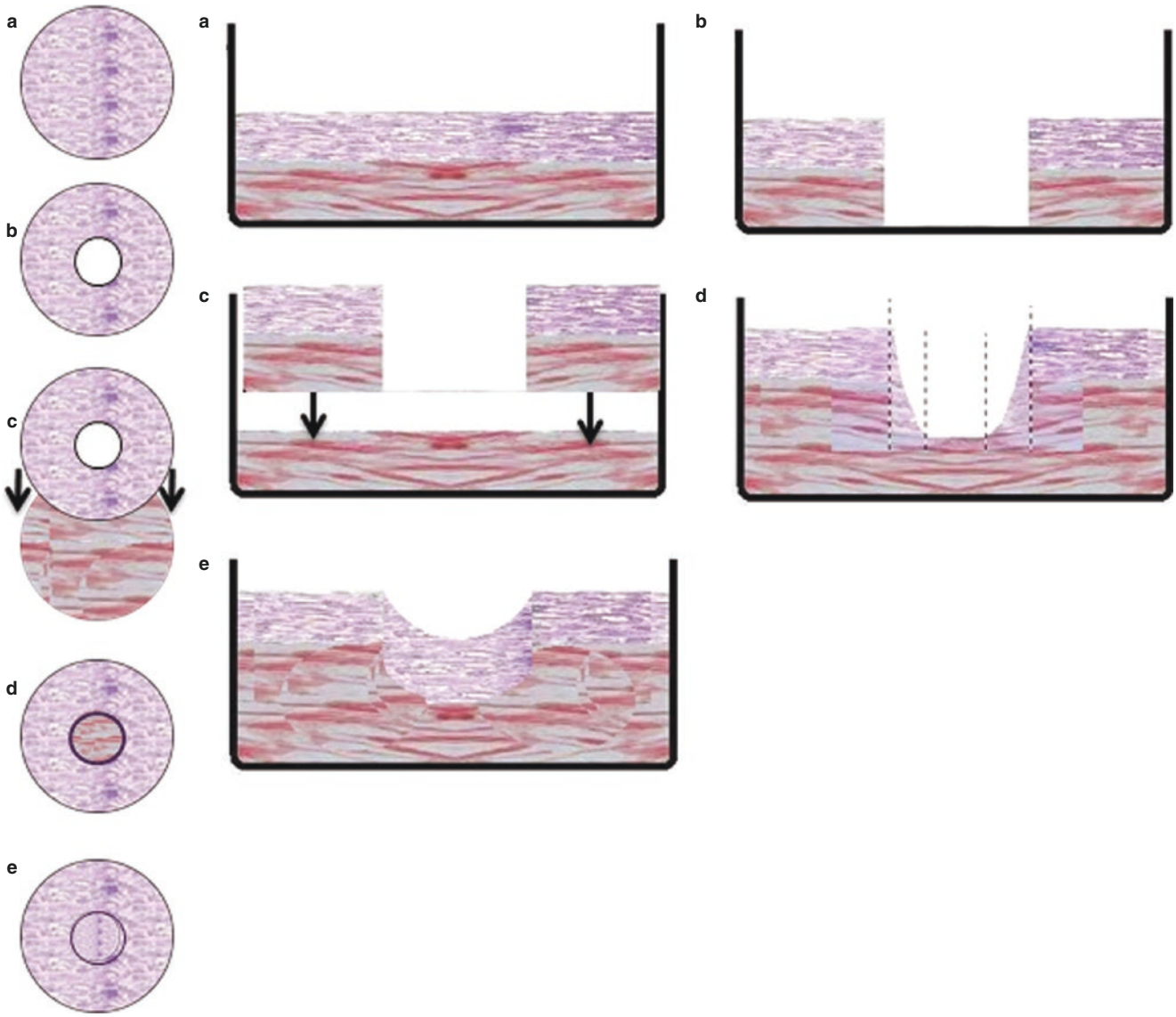

Fig. 13.5 3D skin equivalent tissue model for wound healing. Patient-derived cells and adult or iPSCs can be used to create an in vitro human tissue model of wound healing. HSE tissue wound model is developed through the following sequential steps: (a) HSE tissue is fully developed. (b) A full-thickness wound is made in the tissue using a biopsy punch. (c) Wounded tissue is placed on second dermal layer. (d) Keratinocytes from wounded tissue migrate across the wound bed. (e) Wound is fully reepithelialized and healed. Overhead view is on the left. This tissue model can be used to test how stem cells can stimulate wound healing

In the future, it will be necessary to scale these 3D tissues to high throughput formats to enable the rapid, global screening of tissue responses that mimic DFUs. Development and optimization of DFU-specific, customized 3D tissue models will be prepared with genetic modifications, for example, CRISPR/Cas9, that will enhance the screening of stem cell therapies before they are tested in humans. The DFU research community would benefit greatly by the development of in vitro tools and resources to provide disease-specific and pathway-specific, skin-like tissues that mimic the essential features of DFUs.

Preclinical animal and human studies offer clear evidence that stem cell therapies offer an effective approach for treating DFUs. Studies demonstrating the potential of using adult stem cell sources, including MSCs, HSCs, EPCs, MNCs, and ASCs, now provide a baseline to which the use of iPSCs can be compared as they are developed as a future paradigm for the treatment of DFUs using a single, self-replenishing stem cell source that can differentiate into multiple cell types needed for DFU repair. Modification of both adult and pluripotent stem cells with CRISPR/Cas9 gene editing is another new tool available for improving wound repair of DFUs. To test these the efficacy and safety of these novel therapies, 3D human skin equivalents will provide effective, customizable models that will optimize these new therapeutic approaches.

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MicroRNAs: Novel Therapeutic Targets for Diabetic Wound Healing

14

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Abstract

Diabetic foot ulcer (DFU) represents a major clinical challenge among diabetic complications and combines multiple physiological factors involved in the inhibition of wound healing. Healing of skin wounds is a complex and dynamic process in response to cutaneous injury, which involves a cascade of molecular events orchestrated with temporal and spatial gene regulation in different cell types. Abnormal patterns of tissue repair-related gene expression and resultant cellular malfunctions are key components of impaired healing in diabetic patients. Thus, to understand the pathophysiology of delayed healing in DFU, it is crucial to identify the functional regulators in individual cell types. Recent studies have demonstrated various genetic and epigenetic regulators of the cellular transcriptome and among them highly conserved, tiny noncoding RNAs, especially microRNAs, constitute an important class of master regulators to regulate diverse cellular functions essential for skin wound healing. Here, we will discuss the recent advancements on miRNA regulation of tissue repair processes and their potential as novel therapeutic targets to accelerate healing in diabetic patients.

Diabetic foot ulcers (DFUs) represent a major clinical challenge among diabetic complications and combine diverse physiological factors involved in the inhibition of wound healing [1–3]. Approximately 25% of diabetic patients develop a foot ulcer sometime in their lifetime and more than 60% of these develop into nonhealing wounds despite receiving medical treatment [4]. Skin wound healing is a complex and dynamic process in response to cutaneous injury, which involves a cascade of molecular events orchestrated with temporal and spatial regulation of different cell types [2]. Although it is evident that dysfunction of multiple cell types, including platelets, fibroblasts, keratinocytes, immune cells, and endothelial cells, all contribute to inhibition of healing, our knowledge regarding contributions of individual cell types is still in its infancy [5]. Abnormal patterns of tissue repair-related gene expression and resultant cellular malfunctions are the key components of impaired healing in diabetic patients. Thus, to understand the pathobiology of delayed healing in DFUs, it is crucial to identify the functional regulators of individual cell types. Recent studies have demonstrated various genetic and epigenetic regulators of gene expression and among them noncoding RNAs (ncRNAs), especially microRNAs (miRs, miRNAs), constitute an important class of master regulators controlling the cellular transcriptome, which eventually leads to compromised cellular functions and thereby impairs skin wound healing [6–12]. Here, we will discuss in detail the recent advancements on how miRNAs regulate tissue repair processes and their potential to be developed as novel therapeutic targets to accelerate diabetic wound healing.

Noncoding RNAs: MicroRNA

MiRNAs are part of a vast variate of noncoding RNAs which were once considered to be "evolutionary junk." Until now it is known that only 1–2% of the human genome codes for proteins, meaning that the majority is represented by RNAs without coding potential, usually known as ncRNAs [13, 14]. These transcripts can be detected in most of the tissues and the body fluids. High throughput screenings and expression profiling have revealed the stimuli or stress responsiveness and spatiotemporal variations in ncRNA expression.

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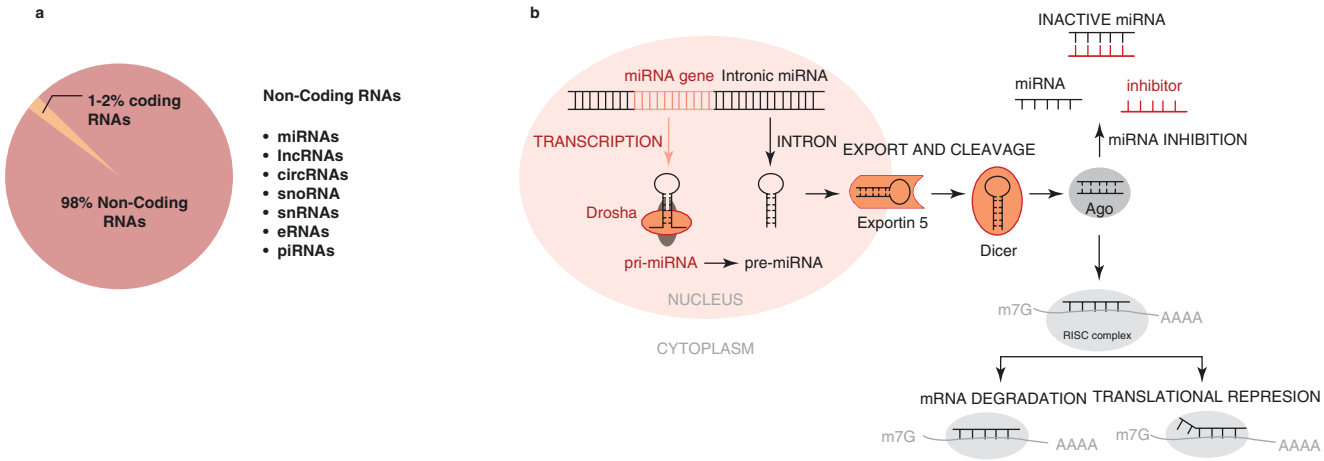

Fig. 14.1 (**a**) Human Genome: 98% is represented by noncoding RNAs, while only 1–2% of the RNAs code for proteins. (**b**) miRNA biogenesis: miRNAs are either derived from introns or transcribed as longer precursors. They mature via endonucleolytic processing and later incorporated into RISC complex to regulate target transcript expression by degradation or translational repression. miRNAs can be modulated either by inhibiting or mimicking their activity

Based on the transcript length ncRNAs can be broadly classified as short or long ncRNAs. Short ncRNAs (<200 nucleotides) mainly comprise well-characterized, highly conserved transcripts of approximately 20 nucleotides in length that regulate gene expression at the posttranscriptional level [7]. The first miRNA, *lin-4*, was originally reported to be essential in developmental events in *C. elegans* [15]. To date, approximately two thousand miRNAs have been mapped to the human genome and with advances in screening tools many more are expected to be discovered [7, 13] . On the other hand, long noncoding RNAs (lncRNAs, >200 nucleotides) lack >100 amino acid classical open reading frame and their discovery is still at a preliminary stage [13, 16]. The linearity of transcripts forms the base of another type of classification and ncRNA transcripts therefore can be classified as linear, e.g., miRNAs and lncRNAs, or circular, i.e., circular RNAs (circRNAs). The 3′ and 5′ ends of linear RNA transcripts join together to make a loop conferring high stability to circular transcripts compared to the linear forms [16, 17]. Due to their high abundancy paired with a high phylogenetic conservation, circRNAs can be potentially exploited for ncRNA-based therapies. However, their functional importance still remains widely unexplored [16].

Genomic origin of miRNAs can be intergenic, that is, from the regions positioned between two protein coding genes, or intragenic, that is, from regions positioned within the coding gene sequences. Initially RNA polymerase-II transcribes them to long precursor RNAs (primary miRNAs: pri-miRNAs). Pri-miRs are then processed in the nucleus and cytoplasm by RNase-III Drosha and other cofactors, resulting in hairpin-shaped pre-miRNAs (~70 nucleotides in length) [18, 19]. Pre-miRs are further shortened in the cytoplasm by Dicer to give rise to the mature miRNA. Once processed from the hairpin [20, 21], miRNAs pair with mRNAs in the Argonaute protein of the silencing complex (RISC or RNA-induced silencing complex) to direct posttranscriptional repression [22, 23] (see Fig. 14.1).

MiRNAs usually bind via complementary base pairing with sequences located in 3′ untranslated regions (UTRs) of messenger RNA (mRNA) [19, 24]. This pairing of miRNA with their mRNA target results in degradation of the mRNA as well as the inhibition of translation, although the vast majority of the gene-regulatory effects of miRNAs occur mainly though target mRNA decay rather than translational repression [24]. Because of the degradation or repression of the targeted mRNA, miRNAs have been implicated in a variety of pathophysiological conditions including wound healing pathology.

miRNA Therapeutics

Due to their causal role in disease pathology, miRNAs have become an innovative therapeutic target. Targeting miRNA function can be classified into two approaches: 1. miR-mimics, to enhance the expression of suppressed miRNAs using a “gene therapy” approach incorporating oligonucleotide sequences coupled with vector carriers such as AAVs (Adeno-Associated Virus Vectors), and 2. the miRNA inhibition, to silence endogenous miRNAs that have enhanced expression causing disease pathology. Although both approaches have been tested in vivo, the most advanced one so far is the silencing of an upregulated miRNA [25].

AntimiRs are antisense oligonucleotides having the complete or partial complementary sequence of a mature miRNA and can reduce the endogenous active pool of the specific

target miRNAs. antimiRs act through steric blocking, a complementary base pairing with miRNAs, which leads to miRNA-antimiR duplex formation with very high stability, rendering the target miRNA inactive [24, 25].

Several modifications have been suggested to improve the efficacy of binding and stability of oligonucleotides used in antimiR design [24]. The first modification that has revolutionized the field of synthetic oligonucleotide therapeutics is the use of phosphorothioate internucleotide linkages at the backbone level instead of phosphodiester bonds. Most of today's used antisense oligonucleotide contains phosphorothioate bonds. Another option is to target the sugar ring [24]. Chemistries with high-affinity 2′ sugar modifications such as conformational restricted nucleotides with 2′-O-methyl (2′-O-Me), 2′-O-methoxyethyl (2′-MOE), and 2′-fluoro (2′-F) have been developed and tested. A modification with an extra bridge connecting the 2′ oxygen and 4′ carbon is called locked nucleic acid (LNA), which has favorable properties and LNA monomers have been successfully applied in various areas of biotechnology and drug development [24].

Cholesterol-Based antimiRs

Cholesterol-based antimiRs, named antagomiRs, conjugated via a 2′-O-Me linkage show full complementarity to the mature miRNA sequence. AntagomiRs contain several phosphorothioate moieties to increase their stability [24]. This class of antimiRs has an enhanced cellular uptake, in particular in the liver [26]. The first silencing of miRNAs using cholesterol-based antimiRs was in 2005. Using intravenous delivery of antagomiRs against miR-16, miR-122, miR-192, and miR-194 resulted in a clear reduction of the corresponding miRNA levels in liver, lung, kidney, heart, and other organs, showing the efficacy of this class of antimiRs [27]. Several other reports on the success of antagomiRs and similar approaches were published after this first attempt.

LNA Oligonucleotides

Further evidence suggests that LNA modifications are superior to cholesterol conjugation antimiRs because they create a thermodynamically stronger duplex formation with the target RNA [28], and although other modifications might improve nuclease resistance, the high duplex melting temperature of LNA oligonucleotides enables efficient miRNA inhibition [24]. An LNA-based antimiR targeting miR-122 was developed to treat viral hepatitis C infections. After successful preclinical testing in nonhuman primates, the drug has already been moved to the clinical phase with promising results in patients [29]. Regarding the cytotoxicity of this chemistry, several studies have reported no hepatotoxicity after LNA delivery in mice, presenting unaltered levels of aspartate aminotransferase (AST) and absence of histological changes in liver sections, showing that besides having a stable chemistry, it has no known side effects [30].

miR-mimics

MiR-mimics are chemically modified exogenous RNA duplexes which may enhance the expression of a particular miRNA upon in vitro or in vivo supplementation. However, this approach has the limitation that the delivery to the target organ is not controlled; therefore, there is a high possibility of off-target effects [25]. Nevertheless, the most popular and effective way of enhancing miRNA function is through adeno-associated viruses (AAVs). It presents an excellent alternative to regulate tissue-specific uptake of the miRNA sequence because of its tropism and low immunogenicity [25]. For example, AAV-6 and AAV-9 display preferential tropism for skeletal muscle and heart when delivered systemically in rodents [31, 32].

All these chemistries, either to mimic or to silence a miRNA, were tested for possible toxic or side effects. In fact, several approaches, for example, nanoparticles and aptamers, were developed to deliver miRNA therapies to specific cell types [33]. RNA aptamers can recognize a specific cell type, and it is also possible to conjugate miRNAs to be delivered to a specific cell. Similarly, nanoparticles can be engineered to target specific cell types; for instance, cells presenting a specific surface epitope can be easily targeted by antibody-coated nanoparticles against the epitope [25, 33].

Skin Wound Healing Regulation by miRNAs

The barrier function of the skin protects living organisms against environmental pathogens. In diabetic patients, the loss of its integrity due to any pathological condition or injury can lead to serious morbidity, lower limb amputations, and lifelong disabilities [34]. Wound repair is a complex process that requires the coordination of a cascade of cellular responses to injury, including inflammation, epithelialization, angiogenesis, and fibroblast proliferation. Abnormal wound repair may lead to an incomplete and irregular regeneration of the skin structures [35].

The repair process of acute wounds in healthy individuals progresses in a linear fashion of cellular and molecular events, whereas chronic wounds of diabetic patients lack the synchronous progression of such events [36]. The inflammatory response usually lasts 48 h to 2 weeks [37], when immune cells identify infecting pathogens and damaged cells, migrate to the area compromised by the injury, and remove foreign particles and cell debris. This phase triggers

a framework to produce new functional barrier after approximately 4 days of injury by creating a permeable barrier (re-epithelialization), forming new blood vessels (angiogenesis) and repairing the damaged skin tissue (fibroplasia) [37].

Subsequent maturation and remodeling of tissue occur for several weeks once the wound is closed. In this phase, extracellular matrix is adjusted and collagen or elastin fiber structure is formed to replace the granulation tissue under control of metalloproteinases [34]. In diabetic wounds, excessive deposition of extracellular matrix proteins causes a pathological condition in patients [36]. Healing of skin wounds is a complex and dynamic process and involves a cascade of molecular events orchestrated with temporal and spatial regulation in different cell types. miRNAs, by regulating functions of these cells, can control skin healing after injury.

miRNAs Modulating Dermal Fibroblasts Functions

Fibroblasts from diabetic foot ulcers maintain unique phenotypes by stimulating excessive proliferation of keratinocytes, showing reduced stimulation of re-epithelialization and angiogenesis, and producing impaired extracellular matrix. Primary fibroblast cells isolated from DFU and healthy nondiabetic individuals have been characterized by using DNA methylation [38], and in 3D tissue models [39]. Several genetic and epigenetic factors were identified by establishing global relation between their transcriptome and miRnome [40]. Global gene profiling studies showed no differences in miRNA and mRNA expression patterns from fibroblasts isolated from diabetic patients' foot when compared to healthy foot fibroblasts [11], but DFU-derived fibroblasts represented deregulation of more than 20 miRNAs in comparison to normal healthy foot fibroblasts [40]. These studies also suggest that altered miRNA expression and impaired cellular functions in DFU fibroblasts is associated with ulceration rather than diabetes.

In this study, Liang et al. identified 331 sets of abundantly expressed and highly dysregulated miRNAs with their direct target genes using an integrative miRNA–mRNA array analysis. Target genes were considered a subset of genes from the expression profiles of DFU-fibroblasts, when genes are differentially expressed, directly targeted by differentially expressed miRNAs and miRNA-mRNA pair expressions are in reverse direction. Among deregulated miRNAs, miR-21-5p, -34a-5p, -143-3p, and -145-5p were identified to mediate diverse effects on cellular functions using predictive regulatory network analysis [40]. These effects are essential for tissue repair, including reduced cell migration and proliferation, induced cell differentiation and senescence, which represent key processes by which dermal fibroblasts support normal wound healing. The study identified 16 miRNA–mRNA parings, out of which 4 were already known and experimentally validated, i.e., miR-21-5p/RECK, SPRY-1, miR-34a-5p/CD-47, and miR-145-5p/IRS-1 [41–43], whereas 12 pairings, namely, miR-21-5p/CD-47, S100-A10, STAT-3, miR-34a-5p/GAS-1, RECK, IRS-1, PDGFRA, miR-145-5p/ABCA-1, GLIS3, PDGFD, PTGFR, and miR-143-3p/PDGFRA were novel. Interestingly, four genes: CD-47, RECK, IRS-1, and PDGFRA were common downstream targets of multiple miRNAs and suggested a synergistic partnership of these upregulated miRNAs [40].

Several identified miRNAs with their target genes contribute to tissue fibrosis which is a key histopathologic characteristic associated with DFUs. A significant increase of miR-21-5p expression in DFU-fibroblasts, and corresponding downregulation of its five target genes, namely integrin-associated protein (CD-47), S100 calcium-binding protein A10 (S100-A10), protein sprouty homolog-1 (SPRY-1), signal transducer and activator of transcription 3 (STAT-3), and reversion-inducing-cysteine-rich protein with kazal motifs (RECK) were confirmed experimentally [40]. miR-21-5p is a known suppressor of RECK and SPRY-1 expression by direct targeting 3′-UTR [43]. RECK is a membrane-anchored glycoprotein which negatively regulates MMPs [44], whereas SPRY-1 is a potent inhibitor of the Ras/MEK/ERK pathway and mediates hypoxia-induced cell death and cell cycle arrest [41].

MiR-145 regulates fibroblast differentiation and TGF-β-induced expression of miR-145/-143 cluster leads to Kruppel-like factor 4 (KLF-4) inhibition, which is a known inhibitor of α-SMA, and hence upregulates α-SMA expression and functions of skin myofibroblasts and DFU-derived fibroblasts [45]. Since myofibroblasts facilitate wound contraction, specifically in granulation tissue during acute wound healing, it is appealing to speculate that the upregulation of miR-145-5p contributes to the deregulation of fibroblast differentiation. Providing that the appropriate execution of the wound healing process is dependent on temporal cellular functions, deregulation of fibroblast differentiation and increased myofiroblast population at the wrong time may prove detrimental to healing. Induction of both miR-21-5p and miR-145 found in DFUFs may contribute to myofibroblast differentiation and tissue fibrosis seen in DFUs [40].

In addition to fibrosis, changes in cell migration and proliferation are known characteristics of DFU-derived fibroblasts. Changes in these cellular functions correspond to increased expression of several miRNAs, including miR-21 and miR-145. Predicted downstream targets of miR-21-5p: S100-A10, STAT-3, and CD-47 are intensively involved in regulation of cell migration and proliferation [40]. In addition, multiple target genes of miR-145 which are also differentially expressed in DFU-derived fibroblasts, regulate cell migration and proliferation. miR-145 is a known suppressor of cell growth by downregulating insulin receptor substrate-1

(IRS-1), one of the key molecules in insulin-like growth factor (IGF)-I and insulin-mediated intracellular signaling [46]. It also targets platelet-derived growth factor D (PDGFD), which acts as a fibroblast mitogen/chemoattractant and stimulates choroidal fibroblast proliferation, survival, and migration [40].

Interestingly, a senescence-linked miRNA, miR-34a, is upregulated in the serum of type 2 diabetes patients and DFU-derived fibroblasts, but remains unchanged in diabetic fibroblasts derived from intact foot skin [11, 47], suggesting that increased expression of miR-34a in DFU-fibroblasts is likely to be associated with ulceration rather than with diabetes. Altered miRNA-mediated gene expression profiles of DFU fibroblasts may be associated with different fibroblast lineages. However, DFU-derived fibroblasts are not yet characterized for any distinct lineage phenotype, the common gene regulations have been observed in DFU-fibroblasts and senescent fibroblasts isolated from wound edge or reticular fibroblasts [48, 49]. Aberrant expression of these three miRNAs (miR-21-5p, miR-34a-5p, and miR-145-5p) may lead to deregulation of DFU-derived fibroblasts and cellular functions essential for effective tissue repair: proliferation, migration, differentiation, and senescence.

miRNAs Modulating Endothelial Functions and Angiogenesis

Angiogenesis is a vital part of the proliferation phase of wound closure and involves the formation of new blood vessels to stimulate granulation tissue remodeling. Excessive surge of proinflammatory cytokines, e.g., TNFα, diminished production of proangiogenic factors, e.g., VEGF, and reduced formation of microvessels are key contributors to impaired tissue repair in diabetic patients [6, 10]. During usual healing processes, expression and functions of these proangiogenic genes are spatially and temporally controlled by inflammatory factors and other gene regulators, which if disturbed can delay tissue repair. Physiological low dose or pulses of TNFα primes endothelial cell sprouting to induce angiogenesis whereas supra-physiological high dose or persistent stimulation of TNFα suppresses angiogenic response [50, 51]. Therefore dermal microvascular endothelial cells are expected to shift to an antiangiogenic phenotype because of prolonged and unresolved inflammatory response in diabetic wounds.

Two recent studies have demonstrated that in addition to fibroblast apoptosis, proinflammatory stressors could exert substantial antiangiogenic effects via increase in angiostatic miRNA expression including miR-200b and miR-191 in endothelial cells [6, 10]. miR-200b is a known hypoxia-sensitive miRNA that induces angiogenesis in hypoxic dermal cells [52, 53]. In diabetic skin wounds, the expression of miR-200b remains higher compared with nondiabetic wounds and the silencing of miR-200b supports wound angiogenesis [10]. Chan et al. reported that injury-mediated transient downregulation of miR-200b when interrupted by in vivo lentiviral delivery of miR-200b impaired wound angiogenesis. Predictive computational analysis suggested globin transcription factor binding protein 2 (GATA-2) and vascular endothelial growth factor receptor 2 (VEGFR-2) to be direct targets of miR-200b, which was further confirmed by target promoter reporter assay and Western blot analysis. Moreover, transient overexpression of GATA-2 or VEGFR-2 in cultured endothelial cells rescued the angiostatic effect of miR-200b and downregulation of miR-200b enhanced GATA-2 and VEGFR-2 expression to activate wound angiogenesis, which was disrupted in diabetic wounds [10].

Promoter sequence analysis and chromatin immunoprecipitation assay revealed that transcription factor p53 can bind and activate the promoter of miR-200b-200a-429 cluster [54]. In line, mRNA expression of this TNFα-sensitive gene, p53, was aberrantly increased in diabetic wounds at day 3 post-wounding [55]. Silencing of p53 resulted in suppression of primary miR-200b expression, suggesting that p53 can stimulate de novo transcription of miR-200b [54]. It is noteworthy that in addition to p53, a functional GATA2-binding site has also been identified in the miR-200b promoter suggesting that GATA2 might serve as a negative regulator of miR-200b expression [56]. In this regard, TNFα-mediated upregulation of miR-200b silences GATA2, which in turn might lead to sustained miR-200b expression due to relieved negative regulation by GATA-2 under conditions of prolonged inflammation.

In addition, excessive TNFα production in type 2 diabetic mice blunted proangiogenic functions of GATA2 and VEGFR2 via miR-200b induction which was reversed by anti-miR-200b strategy in vivo. Neutralization of TNFα in the diabetic wounds improved wound closure and angiogenesis, by correcting both upregulation of miR-200b expression and silencing of GATA-2 and VEGFR-2. Injury-induced repression of miR-200b turned on wound angiogenesis [10].

Plasma levels of stress-sensitive miR-191 are lowered in plasma of diabetic patients which reflects underlying endothelial dysfunction in patients with diabetes mellitus [6, 57, 58]. Modulation in miR-191 expression regulates apoptosis, cell proliferation, migration, and cell cycle under various pathological settings [58]. miR-191 clusters with miR-425, which is conserved in higher eukaryotes [59]. MiRNA array profiles confirm unidirectional changes in circulating plasma levels of both clustered miRNAs from patients with diabetes mellitus with impaired wound healing compared to diabetics without chronic wound.

Current evidences from scientific literature support the existence of extracellular miRNAs-mediated cross-talks between different types of cells under various pathologi-

cal conditions that may exert paracrine effects on cellular signaling and functions [60, 61]. The secretion of miRNAs from vascular lining cells and their transportation as circulating vesicles was shown in context to various pathologies including wound healing [6]. Dermal fibroblasts and microvascular endothelial cells can take up the secreted miR-191 and resulting accumulation of miR-191 in recipient cells in turn mediated detrimental cellular effects such as impaired migratory or angiogenic responses as well as increased apoptosis. miR-191 targets tight junction protein, ZO-1, which is known to increase on the locomotive surface of cells during wound healing [62]. Reduced expression of ZO-1 was observed parallel to miR-191 upregulation in dermal fibroblasts or endothelial cells when cocultured with endothelial cells either maintained under proinflammatory stress or transiently overexpressing miR-191.

In endothelial cells, ZO-1 mediates angiogenesis and its deficiency leads to defects in vascular development with impaired formation of vascular trees important for tissue organization and remodeling [63]. The paracrine regulation of ZO-1 in dermal cells mediated via miR-191 uptake potentially contributes in slowing down of the tissue repair process commonly observed in patients with diabetes mellitus. Collectively, these evidences suggest that high inflammatory stress underlying nonhealing wounds mediates endothelial miR-191 secretion and a subsequent paracrine mechanism involving miR-191 uptake mediated modulation of target gene ZO-1 in recipient dermal cells ultimately compromises the cellular functions essential for tissue repair.

A recent study demonstrated that miR-26a expression is increased in response to diabetic wound injury and local neutralization of miR-26a could improve wound healing by inducing angiogenesis in diabetic mice, partly through SMAD-1 signaling pathway [9]. Notably, miR-26a was also increased in plasma of diabetic patients suffering with chronic wound as revealed by microarray screening of plasma samples in an independent examination of human subjects with type 2 diabetes [6]. High glucose stimulation of endothelial cells markedly increases miR-26a expression. Interestingly, the inhibition of miR-26a rescued impaired growth and migration in the presence of high glucose in microvascular endothelial cells.

This study also explored underlying paracrine mechanism of miR-26a-mediated effects on fibroblasts via its transfer from endothelial cells. Conditioned cultured media collected from microvascular endothelial cells upon transient knockdown of miR-26 significantly improved dermal fibroblast migration, whereas no effects were seen on keratinocytes. In vivo inhibition of miR-26a in diabetic wounds enhanced expression of its target gene SMAD-1. Indeed, miR-26a neutralization of wounds using LNA-based anti-miR-26a increased SMAD-1 expression which was colocalized with CD31-positive endothelial cells in diabetic wounds compared to wounds treated with the scrambled LNA-anti-miRs [9].

The miR-26a-mediated effects were associated with increased proangiogenic BMP/SMAD1-ID1 signaling and robust functional effects on diabetic endothelial cells, but not dermal fibroblasts or macrophage polarization. SMAD-1-associated transcription factor ID-1 can stimulate migration and growth of endothelial cells [64, 65]. Moreover, decreased expression of the cell cycle inhibitor gene p27 was observed parallel to increased expression of ID-1 in diabetic wounds in response to miR-26a inhibition. This strongly suggests that enhanced BMP/SMAD1/ID-1 signaling may serve as the dominant mechanism behind observed increase in granulation tissue thickness and wound closure [9]. These evidences collectively highlight that neutralization of miR-26a favors tissue regeneration predominantly via enhancing endothelial proliferation and angiogenesis.

Another reason of impaired angiogenesis in DFU is endothelial progenitor cell dysfunction but role of miRNA control of endothelial progenitor cell (EPC) function in diabetes has not been reported until recently. Researchers have applied stem/progenitor cell therapy as a potential therapeutic approach for such problems, however clinical outcomes revealed only limited efficacy of patient-derived EPC therapy [66]. Wang et al. reported that lower levels of miR-27b in circulating angiogenic cells from newly diagnosed type 2 diabetes mellitus patients and in db/db mice was responsible for their dysfunction compared to control subjects [4]. Their study concluded that miR-27b mimics can be used to improve angiogenic function of BMACs. miR-27 belongs to the miR-23/27/24 cluster, the members of which are reported to be involved in endothelial function and organ development [67, 68]. In addition to miR-23a/b and miR-24, other angiogenesis-related miRNAs, e.g., let-7f, miR-221, and miR-222, were also detected in diabetic BMACs. However, none other than miR-24 was elevated in diabetic BMACs. It is noteworthy here that miR-24 induces endothelial apoptosis and impairs angiogenesis in myocardial infarction [53] and its effect in wound healing is not yet defined experimentally.

In this study, miR-27b mimic significantly increased proliferation, decreased apoptosis, improved tube formation capacity, and adhesion of homozygous db/db BMAC. In contrast, miR-27b inhibitor significantly impaired proliferation, tube formation, and adhesion and increased apoptosis in heterozygous db/+ BMACs. Supplementing miR-27b improved BMAC function through multiple pathways: (1) via suppressing the antiangiogenic molecules TSP-1, (2) upregulating angiogenic mediators, such as VEGF and stromal cell growth factor-α, and (3) suppressing p66shc, a medi-

ator of mitochondrial oxidative stress. miR-27b also leads to improved tissue repair and regeneration by enhancing the efficacy of diabetic BMAC therapy on wound closure, wound perfusion, and capillary formation [4].

miRNAs Influencing Keratinocyte Functions

Keratinocyte proliferation and migration are essential for adequate re-epitilialization, wound closure, and restoring skin integrity [69, 70]. Genes directly controlling keratinocyte migration including DIAPH1, PLAU, and LAMC2 are post transcriptionally suppressed by miR-198 which results in restricted keratinocyte migration. In addition, deregulation of miR-198 can also decrease fibrinolysis and impair matrix deposition in chronic ulcers.

Sundaram et al. studied spatiotemporal expression of two different gene products from a single transcript. miR-198 is an exonic miRNA located in the 3′ untranslated region of follistatin-like 1 (FSTL-1) messenger RNA which controls FSLT-1 expression upon wounding [71]. FSLT-1 showed pro-migratory effects on keratinocytes whereas miR-198 acted as anti-migratory by targeting a set of genes. TGFβ signaling controlled the fate of the transcript by switching off miR-198 expression and promoting FSLT-1 expression. In nonhealing chronic diabetic ulcers, the failure of this physiological switch leads to persistent expression of miR-198, silencing of FSTL-1 and thereby inhibits keratinocyte migration and delays re-epithelialization. The "see-saw" expression of FSTL1-miR-198 seems to be a unique regulatory switch critical for wound healing, and miR-198 as a potential molecular biomarker for nonhealing wounds [71].

Apart from miR-198, other miRNAs including miR-132, miR-99, and miR-27b also influence keratinocyte functions. Expression of miR-132 is highly enhanced during the inflammatory phase of wound repair as reported in a study by Li et al. which investigated the miRnome of human skin wounds [12]. miR-132 was predominantly expressed in epidermal keratinocytes and peaked in the subsequent proliferative phase. This TGF-β-sensitive miRNAs can regulate a set of genes controlling immune response and cell cycle. miR-132 decreased chemokine production in keratinocytes and their capability to attract leukocytes by suppressing the NF-κB pathway [12]. Conversely, miR-132 increased activity of the STAT3/ERK pathways and promoted keratinocyte growth by targeting heparin-binding EGF-like growth factor (HB-EGF). Moreover, using mice and human ex vivo wound models researchers demonstrated that miR-132 blockade delayed healing, enhanced inflammation, and suppressed keratinocyte proliferation. Taken together, these findings indicate that miR-132 facilitates the transition from the inflammatory to the proliferative phase [12].

Expression of the miR-99 family members was found to be reduced in diabetic wounds and overexpression of this miR family reduced PI3K/Akt signaling and migration and proliferation of keratinocytes, implicating their potential role in the re-epithelialization phase [72]. Similarly, supplementing miR-27b improved keratinocyte migration whereas its inhibition increased keratinocyte apoptosis and mitochondrial ROS. These observations by Hildebrand et al. suggest that miR-27b improves keratinocyte functions and regulates oxidative stress [73].

MiRNAs may also participate in different pathophysiological aspects of immune cells and thereby influence the progress of diabetic wound healing. For instance, miR-155, a miRNA regulating immune response, was found to be induced in diabetic wounds in mice [74]. Deficiency of this miRNA led to a reduced inflammatory response and improved wound closure, an effect associated with increased expression of miR-155 target genes, BCL6, Rho-A [74].

Conclusion

The pathophysiology of DFU is poorly understood which hampers the development of effective treatments. Indeed, diabetic dermal wounds exhibit altered expression of multiple genes leading to compromised cell growth, reduced angiogenesis, irregular matrix protein deposition and therapeutic targeting of these genes can facilitate and accelerate wound healing in diabetes (see schematic Fig. 14.2). In contrast to modulating a single gene at a time, as followed in conventional gene therapy, miRNA-based therapies present a unique advantage of targeting a group of functionally related genes associated with a common signaling or functional pathway, by merely modulating a single common regulatory miRNA. Yet, the multitarget approach may pose a limitation of unwanted side effects and further research is needed to refine this aspect of miRNA therapy.

In addition to providing a platform to develop novel therapeutics, a systematic investigation of miRNA patterns in plasma may reflect on the health of the source cells, presence of stressors or drug treatment and plasma levels of miRNAs therefore may serve as potential biomarkers of various pathologies including diabetes and DFU [6, 57, 75–77]. In fact, the miRNA profiles of blood plasma represent a pool of miRNAs secreted by vascular endothelial cells, blood platelets, and challenged immune cells in the form of exosomes or apoptotic bodies [78–82]. Independent studies have proven the role of various miRNAs in vitro and in vivo diabetic wound healing. Apparently many of them, including miR-191, miR-200, and miR-26a, were also differentially expressed in plasma of diabetic patients with foot ulcers compared to diabetic

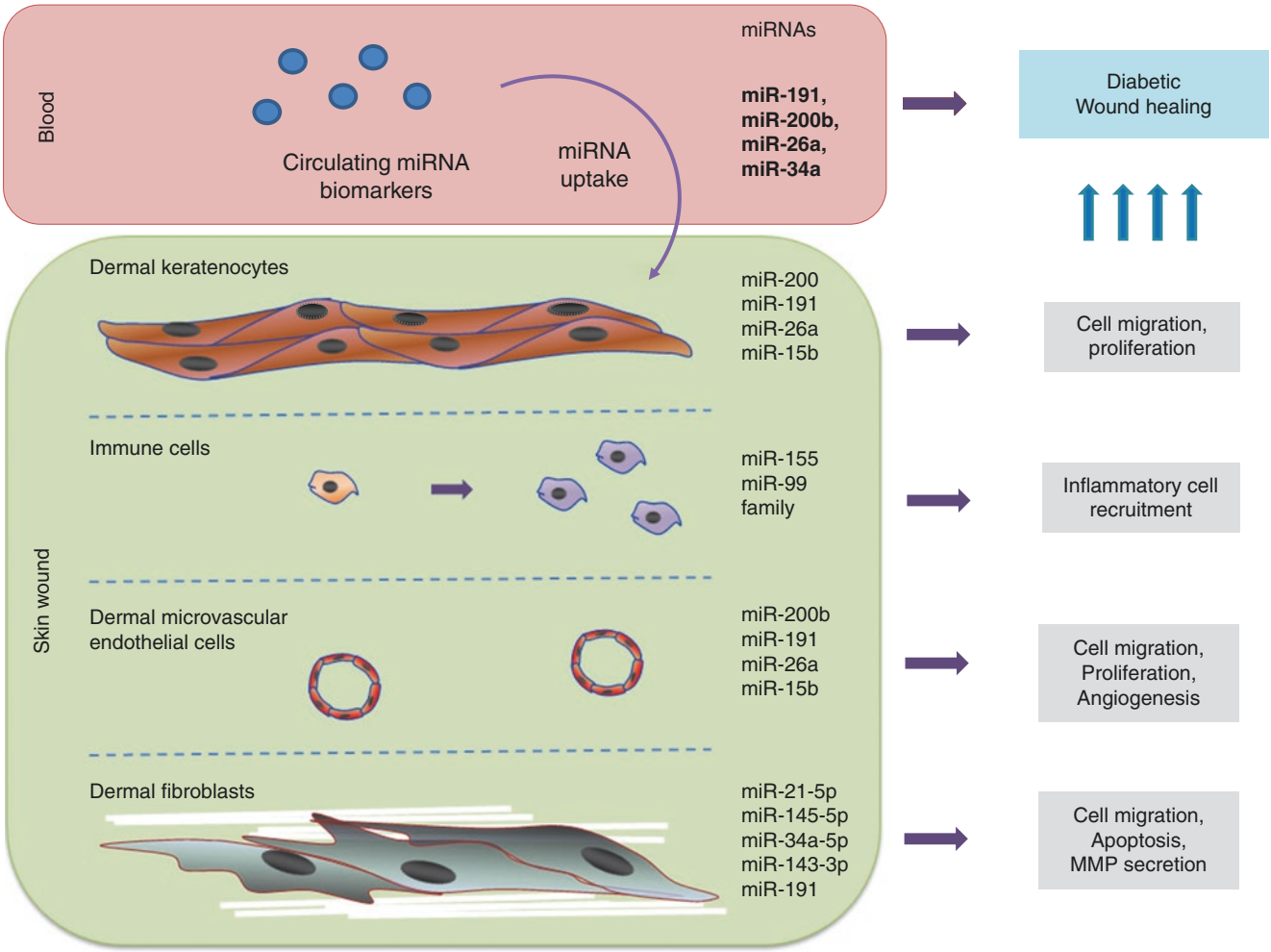

Fig. 14.2 Schematic summary of cell-type specific and circulating miRNAs regulating diverse cellular functions essential for rapid wound healing

controls [6, 9, 10]. Positive correlations were established between plasma levels of both miR-191 and miR-200b to plasma C-reactive protein and cytokine levels which confirmed underlying inflammatory conditions to influence plasma miRNA levels [6].

Recent advances in the synthesis and chemistry of nucleotides have allowed researchers to establish efficient in vitro and in vivo application of miRNA-based therapies. Multiple approaches of miRNA modulation have been successfully used in preclinical and clinical setting [29, 83, 84]. Therefore, miRNA targeting either alone or in combination with conventional treatments may present a novel opportunity to accelerate diabetic wound healing, by rectifying aberrant expression of miRNAs. The recent report on safety and efficacy outcomes from phase-II clinical of anti-HCV drug miravirsen targeting miR-122 is encouraging and suggests the promise of miRNA therapy to treat hepatitis-C infections [85, 86]. miRNA-mediated gene regulation therefore may reveal novel molecular mechanisms of nonhealing ulcers and provide an experimental foundation for the future development of miRNA-based therapies to accelerate tissue repair in diabetic patients.

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Tissue-Engineered Wound Dressings for Diabetic Foot Ulcers

15

Sahar Rahmani and David J. Mooney

Abstract

With the rise in the number of individuals suffering from diabetes, a greater number of patients are at risk of developing diabetic foot ulcers (DFUs). While traditional wound therapies can be successful at treating moderate DFUs when detected early, they often fall short in treating more severe cases which can lead to secondary ulcer formation or lower extremity amputations. To remedy this, a number of FDA-approved therapies have been developed and approved in the past two decades that take advantage of advances in biomaterials and tissue engineering to manufacture materials that have specific functionalities, and exploit active dressing materials that can enhance the wound healing process for these patients. Despite these advances, diabetic patients still suffer from slow healing wounds that often lead to further infections and delayed healing and/or amputations. Recent research in the wound healing field has focused on developing dressings with improved properties, especially the ability to encapsulate and release therapeutics over prolonged durations. These can potentially enhance the wound healing process by controlling cell migration and proliferation into the wound and provide a physiochemical environment conducive to healing. While many of these therapies are still undergoing clinical testing, or have yet to be tested for the treatment of DFUs, they provide a promising future.

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Introduction

Diabetes mellitus is one of the fastest growing chronic diseases worldwide, with an estimated 285 million adults affected by the disease in 2010 [1]. In the United States, the prevalence of the disease was at 12–14% of the adult population in 2012, a number that is expected to grow continuously [2]. Diabetes mellitus is characterized as a metabolic disorder, which presents itself as high glucose levels in the serum, disruption to the metabolism of carbohydrates and lipids, and complications with the secretion of insulin, resistance to the actions of insulin, and/or the inability to fabricate insulin due to the loss of insulin-secreting β-cells [3]. Diabetic patients are likely to develop atherosclerotic macrovascular diseases and diabetes-specific microvascular pathology, especially in the retina, renal, and peripheral nerves, which may consequently result in blindness, end-stage renal disease, and various neuropathies [4, 5]. Diabetic peripheral neuropathy, peripheral vascular disease, and an impaired healing cascade often result in the development of diabetic foot ulcers (DFUs), with a 25% lifetime risk of developing a DFU for a diabetic patient [6, 7]. DFUs are often classified based on their severity using a Wagner Ulcer Classification system, which ranges from superficial diabetic ulcers (Grade 1) to extensive gangrene of the foot (Grade 4) [8]. When untreated, DFUs often lead to lower extremity amputations [1, 9]. In fact, 15% of all DFUs result in a lower extremity amputation, amounting to 85% of all amputations in the USA, at an approximate annual cost of over four billion dollars [10].

While prevention of DFUs, especially using a multidisciplinary team of healthcare professionals (e.g., podiatrist, diabetologist, orthopedic and vascular surgeons, microbiologist, and tissue viability nurses) has been shown to reduce their occurrence rate [6, 8], DFUs are still a major complication faced by diabetic patients. Traditional treatments of DFUs include controlling the patient's glycemic levels (slows the progression of neuropathy), improving vascu-

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larization (increases blood flow to the wound, especially in ischemic legs), debridement of the wound and maggot therapy (removes necrotic tissue for improved healing), offloading (reduces the pressure to the wound), negative pressure wound therapy (removes wound exudates via vacuum to promote healing and promotes regeneration), treatment of infections (local and systemic depending on the severity of the infection), and traditional wound dressings (offers a barrier to contaminants and further injury to the wound) [6–8, 10–14]. Of great importance is the complete debridement of the wound and the presence of a clean wound bed before the start of further therapies, since a lack of full debridement is believed to impede the progress of wound healing [8, 15–17]. While these systems can be effective, the patients still often suffer from amputations or secondary DFUs. A possible route for enhancing the treatment of DFUs might be to use more advanced wound dressings that not only function as a barrier but also take an active role in the healing of ulcers.

One approach for the development of active wound dressings is to address the issue as an engineering challenge, where the properties and characteristics of an ideal therapy are seen as the design requirements and an ideal product is fabricated to meet these specifications. Main design requirements for active wound dressings can be divided into three categories (Fig. 15.1), which include those involved in: (1) material selection, (2) creation and control of the wound environment, and (3) encapsulation and delivery of therapeutics. When selecting a material for wound healing, several parameters should be considered including biocompatibility, immune reactivity, and ease of removal without causing further damage [1]. Once selected, such material should be able to create the proper environment for wound healing and should provide gas exchange, thermal insulation, moisture, drainage of exudates, and antimicrobial capabilities [1, 18]. The material may also be capable of encapsulating and controlling the release of various therapeutics from small molecules to macromolecules and various types of cells [1, 10, 18].

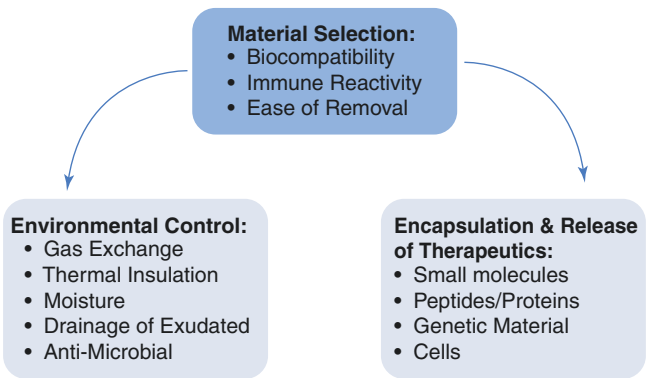

Fig. 15.1 Representative design requirements for the fabrication of successful wound dressings for the treatment of DFUs

Additionally, several cost-related and regulatory aspects must be considered when developing new wound dressings. In order to make these products marketable, and given the high cost associated with the development of such systems, the results need to be considerably more effective than the current standard treatments in order to make them profitable. Moreover, the products need to be user-friendly and require minimum additional training on the part of the caregiver. Lastly, while a number of promising treatments are currently being developed, the long regulatory process required to achieve FDA approval is costly, which must especially be considered for smaller companies [19]. In this chapter, we review some of the current, next generation, and futuristic wound healing systems and discuss their potential with respect to meeting these design requirements.

Current Materials for Wound Healing

There are a number of FDA-approved active wound dressings currently available on the market that are in use for the treatment of DFUs in patients [1, 18]. The materials used for the fabrication of these dressings can be broadly categorized as natural, synthetic, or a combination of both. Natural wound dressing materials are derived from a natural source, and include cellulose, collagen/gelatin, hyaluronic acid, chitosan, and alginate [1, 20]. The natural materials have the advantage of typically being considered biocompatible, have some versatility in mechanical properties, are absorptive, antibacterial, and, certain types, are present as part of the natural healing process [21]. However, their isolation, batch-to-batch variability, processing, and limited range of physical properties can make the use of such material problematic [1, 22]. On the other hand, synthetic materials, including polyvinyl alcohol, polyurethanes, polyesters, and polyethylene oxide/glycol, can be fabricated on a large scale and often inexpensively, are much more well defined with lower batch-to-batch variability, provide a broad range of physical properties, and can be chemically modified to better address various processing and biological aspects [1, 18]. However, the basic synthetic materials often lack some of the inherent capabilities of the natural products and often require further chemical modifications to achieve the desired traits, which can result in higher manufacturing costs. Often, the natural and synthetic materials are combined to take advantage of capabilities from both groups [18].

The currently used wound dressings made from these materials can be categorized into hydrocolloids, hydrogels, foam dressings, films, and skin substitutes, using the terminology common to the field [1, 6, 20]. The common terminology can be somewhat confusing, as hydrocolloids, for example, while by scientific definition a type of material that forms a hydrogel in the presence of water, are typically categorized

in this field as a distinct type of dressing from hydrogels. As wound dressing materials, hydrocolloids are typically composed of an adhesive, film-like dressing and absorbent particulates [1]. These dressings absorb wound exudates to form a gel that protects the wound and creates a moist environment [6]. Unfortunately, there is a limit to the amount of exidates that this type of dressing can absorb, leading to accumulation at the wound site or the breakdown of the wound dressing [8]. Hydrocolloid dressings typically need to be changed multiple times a week to address these issues [23].

Hydrogels are scientifically defined as a broad category of materials comprised of hydrophilic, cross-linked polymers that are typically composed of 30–90% water. In the common terminology used for wound dressing materials, hydrogel dressings are capable of absorbing more liquid than the hydrocolloids, and can create a moist environment for the wound without excess exudates [3, 23]. Hydrogels can be cross-linked ionically or covalently to control their degradation and other chemical/physical characteristics, can be flexible, inert, easily removable, and can allow for gas/liquid/metabolite exchange [1, 24]. While, in the way terminology is used in this field, hydrogel dressings can typically absorb more liquid than hydrocolloid dressings, there is a limit on the amount they can absorb, and in the case of wounds with excess exudates, these dressings will need to be frequently replaced [8].

One way to address wounds with excess exudates is to use foam-type dressings that can absorb a large amount of liquid based on their polymeric makeup and thickness [1, 8]. A foam is technically a type of colloid in which a discontinuous gas phase is distributed in a continuous liquid phase. As this term is used in the wound dressing field, once exudates are absorbed, a foam dressing is considered to exhibit a gel-like texture and creates a moist environment for the wound. Foam dressings can also act as a cushion and a protective layer on the wound, which can enhance their functionality and the comfort level for the patient [1]. This type of dressing can be left on the wound for up to a week due to its excellent absorbance capabilities.

Film dressings are typically transparent, flexible, easy-to-manipulate adhesives that allow for gas exchange, but are non-permeable to liquids and bacterial infections [25]. These dressings are not liquid absorbent and are not typically used with wounds that have more than a moderate amount of exudates [26]. While this might limit their applicability for the treatment of DFUs on their own, their combination with some of the dressings mentioned above can create a more comprehensive system, as they can provide better fixation of the dressing to the surrounding tissue and create a barrier for liquid transport and bacterial infections [1].

Perhaps the most advanced dressings that are currently in use for the treatment of wounds are skin substitutes [11, 20, 21]. These are typically composed of natural or synthetic scaffolds that have been seeded with various cells involved in the wound healing process, especially fibroblasts and keratinocytes. These scaffolds provide cellular support and structural integrity to aid in the wound healing process [23]. The cells incorporated in these scaffolds are allogenic [20], and while they typically do not persist in patients for more than 6 weeks, they do tend to be clinically more effective as compared to their noncellular counterparts [27–31]. This is in part attributed to the secretion of cytokines, proteoglycans, growth factors, and ECM components by the cells, which are vital in the proper and non-chronic wound healing cascade [32, 33]. A number of these skin substitutes are FDA approved to treat DFUs, and have been shown to improve the rate of wound closure [34, 35]. Unfortunately, this type of wound dressing on its own is not capable of absorbing exudates and will require an additional dressing for this function. Additionally, they typically lack dermal structures, such as sweat glands and hair follicles [36]. While further side-by-side studies are needed to properly compare the effectiveness of different types of wound dressings for the treatment of DFUs, and other factors such as cost and availability must also be considered, skin substitutes open the door for more advanced wound treatment options that can take a more active role in enhancing wound healing in diabetic patients. Table 15.1 outlines a representative sample of current FDA-approved therapies from each of these categories and outlines their significant capabilities. A more complete list of the current therapies has been published in a number of recent reviews [16, 37].

The current set of FDA-approved therapies are certainly an improvement over traditional therapies, especially with respect to the design parameters that were outlined earlier in this chapter. The majority of the materials outlined here are biocompatible, do not elicit a harmful immune response, and can be easily removed without further damage to the wounds. Additionally, they can provide gas exchange, thermal insulation, a moist environment, and provide a barrier to infections. The different materials perform variably with respect to the absorption and drainage of exudates from wounds, with foams capable of absorbing the largest amount of liquids, followed by hydrogels, hydrocolloids, and films. Since DFUs have differing amounts of exudates depending on their severity and the stage of healing, this provides the caregiver with a range of options to choose from, but it does not guarantee a one-size-fits-all mentality. Additionally, while skin substitutes provide a means for the delivery of various therapeutics to the wounds via secretions from their loaded cells, few of the other FDA-approved wound dressings contain therapeutics to enhance healing. As such, while the current wound dressings address some of the design requirements for an ideal system (material selection and control over the environment), they often fall short in the therapeutic encapsulation and release category, a next step in enhancing wound healing in diabetic patients.

Table 15.1 A representative list of FDA-approved wound dressings for the treatment of DFUs

Dressing type	Product	Company	Description	Represen. references
Hydrocolloids	Aquacel	ConvaTec	Antimicrobial hydro-fiber with carboxy-methyl cellulose and ionic silver	[38–42]
	Comfeel	Coloplast Corp	A semipermeable polyurethane film embedded with calcium alginate and carboxymethylcellulose particles	[11, 43]
	DuoDerm CGF	ConvaTec	A semipermeable polyurethane foam dressing	[44, 45]
Hydrogels	Restore	Hollister Woundcare	Calcium alginate dressing with silver lining for antimicrobial properties	[46–48]
	Carrasyn	Medline Industries	Hydrogel containing aloe vera for simple hydration of wound site	[11, 49]
	Purilon	Coloplast Corp	Hydrogel composed of calcium alginate and carboxymethylcellulose	
Foam dressings	Tielle	Johnson & Johnson Medical	A semipermeable, thin sheet of hydrophilic polyurethane with acrylic adhesive coating	[50–52]
	Allevyn	Smith & Nephew, Inc.	Combination of polyurethane foam and films, with 5% silver sulfadiazine. Contains an acrylic adhesive that aids in easy removal of the bandage.	[53–55]
	Lyofoam	Seton Healthcare Group, PLC	A semipermeable polyurethane foam sheet	[56, 57]
Films	OpSite	Smith & Nephew, Inc.	A thin, semipermeable polyurethane foam sheet with an acrylic adhesive coating.	[58, 59]
	X-Cell	Medline Industries	A non-adherent cellulose dressing with polyhexamethylene biguanide for broad spectrum antimicrobial function.	[25, 60]
	Tegaderm	3 M Healthcare	A thin, semipermeable polyurethane membrane with an acrylic adhesive coating	[61, 62]
Skin substitutes	Apligraf	Organogenesis, Inc.	Bovine type I collagen scaffold with human fibroblasts and keratinocytes	[63–65]
	Dermagraft	Organogenesis, Inc.	Bio-absorbable vicryl mesh with neonatal foreskin fibroblasts	[28, 29, 66, 67]
	Epifix	MiMedx Group Inc.	Human amniotic membrane with epithelial cells	[65, 68, 69]

Next Generation Materials for Wound Healing

To address some of the challenges faced by the FDA-approved wound dressing systems, current research is focused on developing materials that can address their physical/chemical shortcomings as well as to provide the ability to deliver therapeutics, including peptides, proteins, genetic material, naturally derived agents, and various cell types [8, 10, 18]. While the majority of these systems have not been tested in humans and/or are currently in the early stages of clinical testing, or they have been tested for general wound healing and not specifically for treating DFUs, there is certainly a number of promising materials that could be used to enhance healing and wound closure in diabetic patients.

A number of the currently developing wound care systems use the same natural and synthetic materials discussed earlier in this chapter. However, what makes these systems often unique is their altered chemical structures, which enhance their capabilities. Whether these alterations result from the blends of multiple polymers, copolymerization of two or more different polymers, or the modification of monomers/polymers to add functional groups, these chemical changes result in products intended to better meet the design parameters for creating a more effective therapy, such as their biocompatibility/degradability, inertness, swelling ratios, and adhesiveness, to name a few [24]. Additionally, these modifications can enhance the encapsulation of therapeutics and create better control over their release profiles. As an example, alginate is a natural polymer that is traditionally used as a wound dressing by cross-linking it via cations to form alginate hydrogels. Research in various groups in the past decades have resulted in a number of different modifications to the alginate polymer or how it is used to fabricate hydrogels, ranging from providing covalent cross-linking that enhances durability and improves mechanical characteristics to modifying the polymer to include peptides or signaling molecules to enhance the loading and delivery of cells [24, 70]. Modified polymers, in combination with the appropriate payload, might be an avenue for the fabrication of ideal wound dressings for the treatment of DFUs that meet all of the required design requirements.

Building on previous successful cell delivery systems, numerous research groups are focused on expanding the range of cells delivered or their delivery vehicles [16]. While extensive research has been conducted in the delivery of fibroblasts and their role in regeneration [71, 72], a number of studies have instead focused on the delivery of stem cells to treat wound healing [73]. Mesenchymal stem cells have been shown to accelerate wound healing in DFUs and prevent the formation of secondary ulcers by controlling the inflammatory phase, providing a well-vascularized

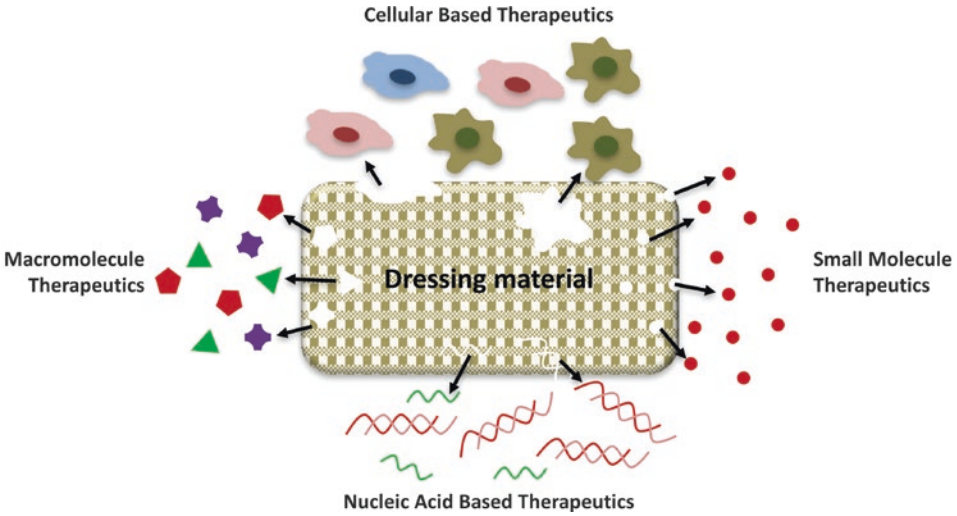

Fig. 15.2 The encapsulation and release of therapeutics from wound dressing materials

environment, attracting keratinocytes, and preventing apoptosis of cells involved in the wound healing process [16, 74, 75]. While some studies have applied the cells directly to the wound [73], others have used various materials, such as fibrin, to deliver the cells [74]. A number of these strategies are currently in various phases of the regulatory approval [76]. Additionally, other cell types have been incorporated into skin substitute products to enhance wound healing, including melanocytes to improve the aesthetic outcomes [77], endothelial cells to improve blood supplies and lymphatic drainage [78–80], and eccrine sweat gland cells to enable sweating and the creation of a moist environment for healing [81].

In addition to delivering cells, a number of studies have shown a significant improvement in the healing rate of DFUs by delivering various therapeutics (Fig. 15.2). Traditionally, such therapeutics are applied topically either in liquid form or as a more viscous ointment during the placement of dressings on wounds. However, due to the large amount of exudates leaving the wound in most DFUs, the short life span of many biological therapeutics, and the need for a continuous presence of these therapeutics to be clinically applicable, their topical delivery is often not successful [82]. To address this challenge, many of the current wound healing systems under development take advantage of research in the drug delivery field to incorporate therapeutics into their wound dressings, which allows for the protection of the encapsulated therapeutic over longer durations and their controlled release over a more clinically relevant period. As demonstrated in Fig. 15.2, the encapsulated therapeutics include a wide variety of substances such as macromolecules, cells, small molecules, and nucleic acid based therapeutics that are encapsulated in the dressing material and released over time in the wound environment to enhance the therapeutic effect of the dressing. Here, we review different wound dressings loaded with various therapeutics ranging from small molecules to various macromolecules that are currently under investigation for use in wound healing.

Some of the smaller molecules used for the treatment of DFUs, including iodine, phenytoin, nitric oxide, curcumin, and ciprofloxacin, have been successfully delivered using a number of material systems including polyvinyl alcohol, various polyesters, polyvinyl pyrrolidone, alginate hydrogels, and hyaluronic acids to aid in the healing of the wounds as well as to provide an antimicrobial environment [1, 83–88]. Peptides, proteins including growth factors, DNA plasmids, and siRNAs are among the macromolecules that have been explored to enhance wound healing. A number of peptides and proteins have been incorporated into scaffolds to aid in the adhesion, migration, and proliferation of pro-healing cells into the wound bed, including integrin-binding peptides [89], laminin [90–92], fibronectin [93–95], and fibrin [96]. The role of growth factors in aiding wound healing is well established [8, 11, 72, 97], and numerous studies have used materials to deliver these potential therapeutics to improve wound healing [98–105]. Growth factors, such as FGF [106–108], EGF [99, 109], TGF-β [110], KGF [111], and PDGF [100], have also been incorporated into skin substitutes to successfully promote healing. In the area of gene therapy, various nucleic acid-based therapies, such as DNA plasmids [112, 113], siRNAs [114], and adenoviruses [115, 116], have been explored as therapies, as these act by effecting the expression of target genes responsible for wound healing and regeneration [82, 116–120]. Additionally, a number of natural substances that are a combination of amino acids, enzymes, vitamins, and polysaccharides have also been delivered using wound dressings and have been reported to improve wound healing in diabetic wounds. These natural substances include honey [121, 122], aloe vera extract [123, 124], essential oils [125, 126], and plant extracts [127].

Present research in tissue-engineered materials for wound healing addresses some of the shortcomings faced by the

currently FDA-approved products. These new technologies aim to meet the design specifications with regard to the material selection (biocompatibility, immune reactivity, and ease of removal), environmental control (providing gas exchange, thermal insulation, moisture, drainage of exudates, and antimicrobial capabilities), and, specifically, the ability to encapsulate and control the release of various therapeutics from small molecules to macromolecules and various types of cells. Materials able to deliver a wide range of therapeutics might be best suited to create the next generation of therapies for wound healing in diabetic patients since they could impact a wide array of key features, including cell migration and proliferation into the wound, and can have control over the physiochemical environment by presenting pro-healing agents rather than inflammatory ones. Key challenges that still need to be addressed, though, include the fabrication of materials that can both absorb a large amount of exudates and release therapeutics in a controlled manner. Hydrogels, for example, swell as they absorb liquids and this may lead to a burst release of the encapsulated therapeutics instead of their controlled release over time. The incorporation of multiple therapeutics that are diverse in their chemical makeup (e.g., simultaneous delivery of small, hydrophobic molecules and hydrophilic, growth factors in the same wound dressing) is another challenge. A possible solution might be the use of materials that have multiple functionalities or compartments, or are fabricated using a combination of materials. However, in doing so one must always consider the loss of capabilities of each material when combined together. Perhaps more pressing is that while all of the current therapies mentioned in this section have been explored for wound healing, not all of them were necessarily used for the treatment of DFUs. As such, further studies are required to demonstrate their viability in diabetic patients.

Future Directions in Wound Healing Material Systems

In order to develop the next generation of treatments, it may be useful to take a step back and take a fresh look at the pathophysiology of the disease as well as the current and potential therapies. While current therapies in the field focus on creating more advanced versions of the current wound dressings, it might be possible to think outside the box and create a new set of technologies that manage the treatment of DFUs via alternate approaches, rather than adding to the large library of wound dressings that at times differ only slightly from each other. In this final section, we take a look at some of these potential alternatives.

In terms of material and engineering desing, a possible approach to treating DFUs is the use of particulate matter rather than dressings. A number of therapeutic-loaded nanoparticle treatments are currently under investigation for the treatments of DFUs, with many of them demonstrating better wound closure than traditional therapies [128–131]. While this is promising, nanoparticles face longevity issues in the wound environment, especially due to the large amount of exudates in certain DFUs. A possible remedy may be the use of targeted nanoparticles that can be quickly up taken by target cells. This could provide an opportunity to deliver therapeutics to specific cells in the wound environment and the ability to differentially affect the response of various cells to enhance wound healing more comprehensively.

Looking at the pathophysiology of wound healing, there have been a number of new research advances relative to the role of the immune system in wound healing, especially in diabetic patients. In healthy individuals, the immune system has a pivotal role in various steps of the wound healing process and plays an important role in the regulation of various growth factors, cytokines, and molecules involved in the body's response to injury [132]. While it is well known that diabetic patients suffer from an impaired immune system, and that this often changes their response to wound healing, the exact mechanisms involved in the diabetic foot are often unclear [133, 134]. As such, it might be useful to approach the issue of wound healing by addressing the impairment in the immune response, rather than the downstream effects this has on the healing process.

Another aspect to consider is the diversity seen in diabetic foot ulcers and their specific needs. As mentioned previously, DFUs are classified based on the stage of their severity via the Wagner system, but this does not address the different stages as they heal, or the differences seen in patients as a function of their underlying pathologies. Fabrication of adaptive or "smart" materials that can sense changes in their environment and adapt via chemical/physical changes might allow one to address some of these issues (e.g., the varying amounts of moisture or therapeutics needed at different stages). However, this does not necessarily address the full spectrum of challenges related to patient diversity. Perhaps this requires more personalized therapy systems that are specific to each individual's needs. Currently, personalized medicine is often considered too costly, but this might be addressed if one could develop strategies that enable personalized treatments at lower costs.

Conclusions

As the number of individuals with diabetes grows each day, a greater number of patients are at a risk of developing diabetic foot ulcers. When detected in the early stages, the treatment of DFUs can be accomplished without further complications, but when left untreated can lead to lower extremity amputations, which can have a profound effect on the lives of individuals suffering from the disease. While a number of traditional therapies exist for the

treatment of DFUs, they often fall short in completely healing the wound. Past advances in the field of tissue engineering have resulted in a number of FDA-approved wound dressings that address some of these issues and have resulted in the improvement of treatments for diabetic patients. Furthermore, new advances in the field have led to a number of potential treatments that take a more active role in the healing process, and the next several years will determine their efficacy as they undergo a prelude to regulatory approval. Looking into the future, it will be important to continue the work in this field and perhaps broaden the scope to develop therapies that use a diverse range of technologies, more thoroughly study the underlying issues that lead to the impaired healing in diabetic patients, and create more personalized strategies for individuals while keeping costs low. In the past several decades this field has seen a dramatic change in the way it has addressed wound healing, and equally or more impactful changes are anticipated as the field heads into its next transformative phase.

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Preparation of the Wound Bed of the Diabetic Foot Ulcer

16

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Abstract

Diabetic ulcers are chronic wounds which, despite recent advanced therapies, still fail to heal; they result in infection and high amputation rates. Hyperglycemia induces the majority of micro- and macrovascular complications associated with impaired wound healing. Wound bed preparation (WBP) is an essential step of diabetic wound management in order to accelerate endogenous healing and/or facilitate the effectiveness of other therapies. The aim of WBP is to remove the barriers that impair wound healing, including the presence of necrotic tissue, senescent cells, altered extracellular matrix, hypoxia, high bacterial burden, and inflammatory enzymes within the wound bed. There are several steps for achieving WBP, including debridement, reduction of the bacterial burden, management of edema and exudate, and correction of resident cell abnormalities. Here we provide an overview of the current status, role, and key elements of WBP in the context of diabetic ulcers. We will also introduce a reappraisal of WBP.

Abbreviations

bFGF	*Basic fibroblast growth factor*
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EGF	Epithelial growth factor
EPCs	Bone marrow-derived endothelial progenitor cells
FDA	Food and Drug Administration
HBOT	Hyperbaric oxygen therapy
iPSCs	Human induced pluripotent stem cells
M1	Macrophages proinflammatory phenotype
M2	Macrophages anti-inflammatory and proheal-ing phenotype
MMP-9	Metalloproteinase-9
MSC	Mesenchymal stem cells
PDGF-BB	Platelet-derived growth factor BB
PRP	Plasma rich in platelets
rhEGF	Human recombinant epidermal growth factor
TGF-β	Transforming growth factor beta
TIME	Necrotic Tissue, Infection/Inflammation, Moisture balance, healing of Edge of wound
VEGF	Vascular endothelium growth factor
WBP	Wound bed preparation

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Impaired Wound Healing in Diabetic Ulcers

The healing of a wound requires a well-organized integration of the complex molecular and biological events of cell proliferation, cell migration, and extracellular matrix (ECM) deposition and remodeling. Cellular responses to inflammatory mediators, to cytokines and growth factors, and to mechanical forces must be appropriate and precise. However, in wounds with preexisting pathophysiological abnormalities (chronic wounds, such as diabetic ulcers), evolutionary adaptations have probably not occurred; impaired healing is the consequence.

The fundamental biological and molecular events after cutaneous injury has been described as a complex and dynamic multistage process which involves the reconstitution of the dermal and epidermal layers of the skin and which progresses through four overlapping phases: coagulation, inflammation, migration-proliferation (including ECM depo-

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sition), and tissue remodeling [1]. However, these stages may not be totally applicable to chronic wounds [2]. Clinical and experimental evidence suggest that diabetic ulcers and other types of chronic wounds do not follow an orderly and reliable progression of wound healing [3, 4]. Other factors are involved in impaired healing. For example, chronic wounds are characterized by resident cells that have undergone phenotypic changes that need to be corrected for optimal healing to occur [5].

Coagulation is needed for hemostasis and wound protection. Platelets release a wide range of growth factors and other mediators to help in cell recruitment and later ECM formation. In diabetic patients hyperglycemia can cause increased platelet reactivity and platelet activation [6]. The recruitment of neutrophils and macrophages helps in wound debridement; however, their function is impaired in diabetes [7]. Also, diabetes is associated with oxidative stress and inflammation [6]. Several studies have shown that in diabetes wound macrophages transition from a proinflammatory (M1) phenotype to an anti-inflammatory and prohealing (M2) is defective [8]. Moreover, macrophages show a decrease in the release of cytokines and other mediators. Increasing serum levels of inflammatory cytokines, Metalloproteinase-9 (MMP-9), and the inappropriate response to several growth factors may be responsible for diabetic ulcers' failure to heal [9]. Growth factors mediate the cellular interactions among a variety of cells that orchestrate the wound healing process. Transforming growth factor beta (TGF-β) is a key factor throughout the wound healing process. However, cells in chronic wounds may not respond to TGF-β [10, 11].

A typical feature of chronic wounds, including diabetic ulcers, is their propensity to become highly colonized with bacteria. Even during the normal process of wound healing infection complications can occur [12, 13]. Infection complicates almost 50% of diabetic ulcers, and it is associated with significant morbidity, and may lead to amputation [14, 15]. Bacteria can thrive within the wound as multilayered microbial colonies, known as biofilms, surrounded by a protective coat of polysaccharides. Biofilm is resistant to antimicrobials and contributes to persistent infection and delayed wound healing [16–18]. Specific microbiome might be associated with diabetic wounds and that could impact the capacity to effectively manage these ulcers [12, 14, 16, 19]. Better understanding of the diabetic ulcer microbiome and how these bacteria interact with one another and the host may be crucial to find new strategies to effectively control the growth of polymicrobial biofilms, control the infection, and improve healing [12, 19]. Moreover, pressure may favor overgrowth of bacteria [20, 21]. Vascular abnormalities play an essential role in the rapid spread of infection in diabetic ulcers. Together with hyperglycemia and other metabolic effects of diabetes, tissue hypoxia adversely affects neutrophil and macrophage function [22].

During the migration-proliferation and remodeling phases wound contraction begins. Angiogenesis, the formation of ECM proteins, contraction, and keratinocyte migration are essential components of these phases. The balance between contraction and keratinocyte-dependent wound closure has much to do with the depth and location of the wound, and seems to be impaired in diabetic wounds [7]. Moreover, keratinocyte migration and proliferation may well be impaired in diabetic wounds because of hyperglycemia and chronic inflammation in the wound bed [23]. However, the events leading to neovascularization and wound contraction also play an important role in both acute and chronic wounds [24, 25]. Endothelial cells need to be rapidly activated and migrate to distant sites. There, they proliferate to form new vascular channels from existing ones in response to angiogenic stimuli [26]. However, in a wide range of disease states, including diabetic wound healing, angiogenic responses are impaired [7].

Another critical balance is the deposition and dynamic remodeling of the ECM. Excessive deposition of some ECM proteins, including collagens and fibronectin, has been reported in diabetic wounds [5]. Thus, remodeling or turnover of ECM might be inadequate, which ultimately affects cell migration and probably the stability of the healed wound. Metalloproteinases (MMPs) and other enzymes are important components of the wound and facilitate cell movement and the eventual remodeling of ECM. Directly or indirectly, hyperglycemia alters the balance of MMPs concentrations; excessive proteolytic activity affects patients with diabetic ulcers [27]. Excessive release and activation of some MMPs, such as MMP-9 [9, 27, 28], play a role in various chronic nonhealing wounds, including diabetic foot ulcers, and can impair cell migration and lead to breakdown of some essential ECM proteins and growth factors [29]. Also, diabetes is associated with reduced concentrations of urokinase plasminogen activator and increased tissue plasminogen activator inhibitor, which might result in decreased fibrinolysis and impaired ECM deposition [7]. Failure of timely and rapid contraction seems to be a major problem in diabetic ulcers [7].

There is also increasing evidence that some of the resident cells in diabetic ulcers become phenotypically altered, which may impair their capacity for proliferation and movement. However, these phenotypic changes in chronic wound cells might not be due only to cell senescence or their capacity to response to growth factors but also cause by more complex interactions between the wounds cells and the chronic wound microenvironment [30, 31].

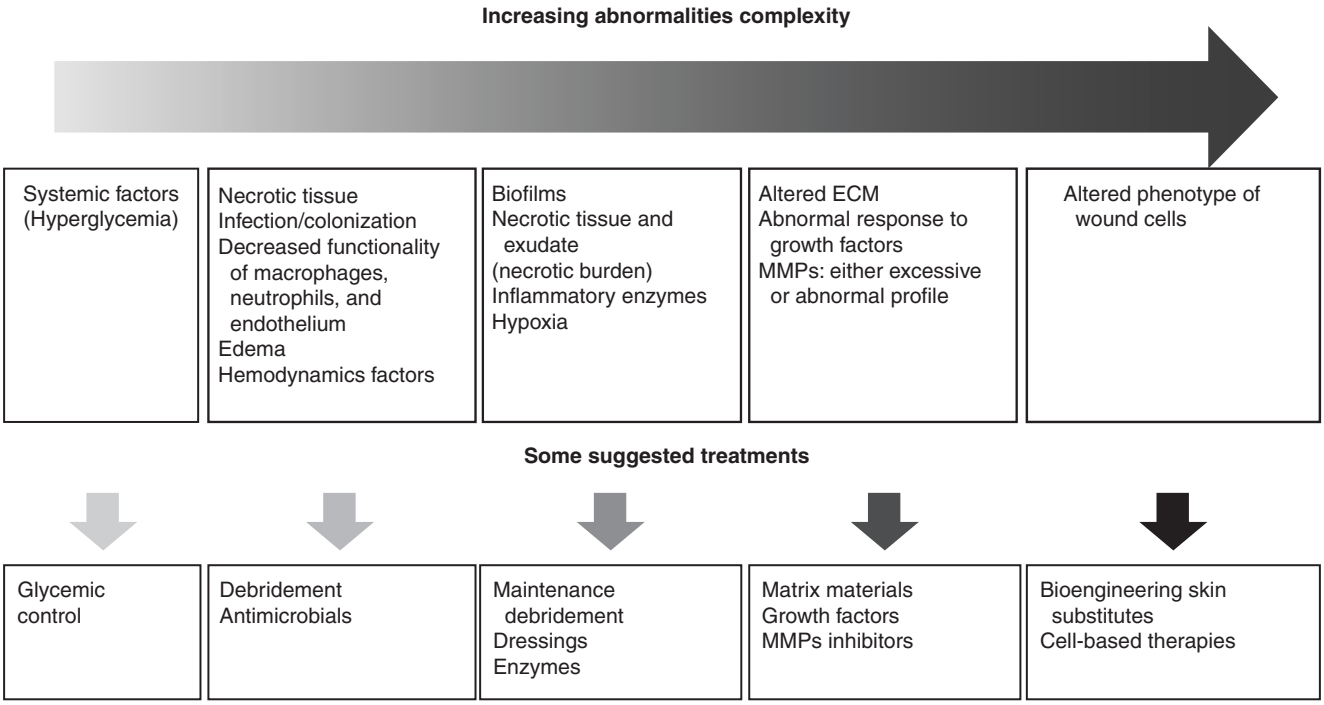

Fig. 16.1 Wound bed preparation: removal of barriers to healing

Wound Bed Preparation

A major advance in addressing the approach to chronic wounds has been the concept of wound bed preparation (WBP). The aim of WBP is to convert the molecular and cellular environment of a chronic wound bed into a normalized acute wound healing process [32].

The aim of WBP in the context of diabetic ulcers is to remove the barriers delaying wound healing. Such barriers include necrotic tissue, altered ECM, senescent wound cells, the bacterial burden, tissue hypoxia, and inflammatory enzymes within the wound bed [32, 33]. Moreover, by implementing WBP, the formation of healthy granulation tissue will be improved and the efficiency of biological therapies optimized.

There are several steps in WBP, including debridement, elimination or reduction of bacterial load, management of edema and exudates, and correction of the cell abnormalities within the wound, together with systemic corrections [34] (Fig. 16.1). Localized and systemic approaches include tight glycemic control, edema improvement, use of off-loading, control of the patient's nutritional status, and smoking cessation [7].

Role of Debridement

Debridement is an important part of WBP. However, debridement alone is not enough to sustain healing in chronic ulcers. In accordance with the TIME (necrotic Tissue, Infection/Inflammation, Moisture balance, healing of Edge of wound) principles, debridement can help remove necrotic burden of abnormal or senescent cells, fibrous tissue (eschar), and control inflammation or infection, decrease excess moisture, and stimulate a non-advancing wound edge [35, 36]. By removing diseased tissue, appropriate debridement of diabetic ulcers corrects several cellular (altered resident cells) and molecular (matrix material, growth factors, MMPs, and enzymes) abnormalities [37] (Fig. 16.2). One hypothesis is that debridement resets the stage for proceeding towards the normal wound healing sequence [7].

In the early stages of wound healing, debridement occurs autolytically through the action of neutrophil-derived enzymes. Protease inhibitors are also released by wound cells to restrict protease's action to the wound bed, and minimize damage to intact tissue at the wound edge. Although debridement may occurs naturally and is often all that is required to promote the first step in the healing process, active debridement is almost always required in the management of diabetic ulcers [38, 39]. Early debridement can accelerate the time to heal, but a program of maintenance debridement is often needed to keep the wound in a healing mode [40, 41]. Maintenance debridement in between surgical interventions may be achieved by several methods such as mechanical, autolytic, chemical, or biological [32, 36]. However, the relative efficacy of these methods in the diabetic ulcers management is not well established [37, 42]. Generally, mechanical means of debridement are required.

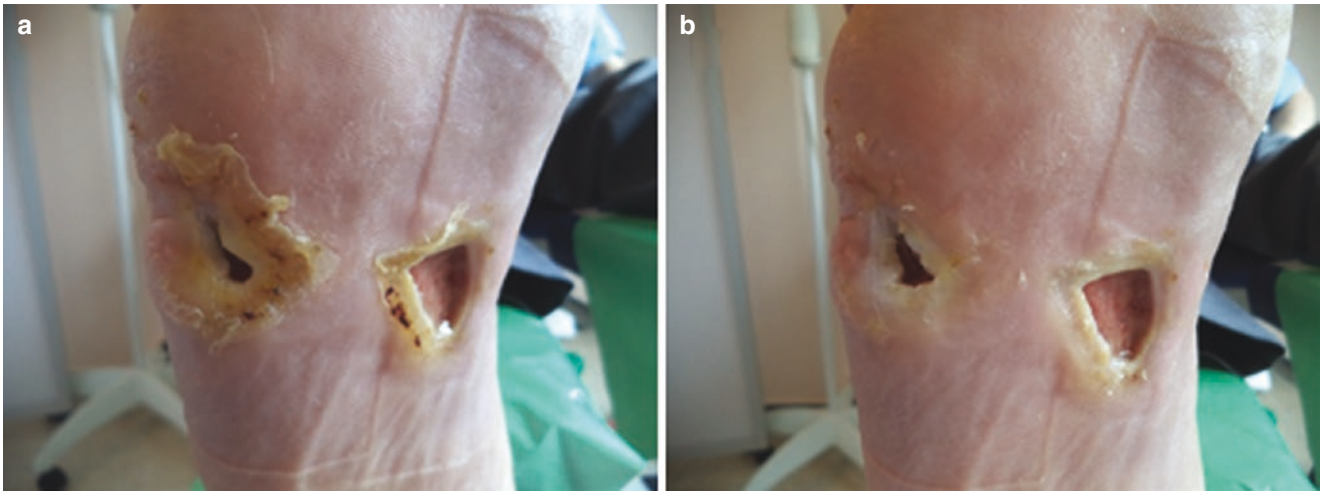

Fig. 16.2 Callus around neuropathic diabetic wounds. (**a**) Ulcer surrounded by an extensive and thick callus, (**b**) the callus around the wound has to be removed

Extensive wound debridement is a critical step for managing diabetic ulcers [15, 38]. Surgical debridement of diabetic foot ulcers not only removes necrotic tissue but also remove the excessive bacterial burden and maybe the phenotypical abnormal resident cells of the wounds [5, 37]. Mechanical debridement is rapid, but nonselective, and is used for wounds with larger amounts of necrotic tissue [32]. Hydrosurgical debridement is a selective, efficient, and rapid debridement method [43]. Autolytic debridement occurs by using the body's own endogenous proteolytic enzymes and phagocytic cells in clearing up necrotic debris. This process is facilitated by the use of moisture-retentive dressings and may take weeks [44]. In our opinion, dressings' approach is generally not acceptable in diabetic ulcers. Enzymatic debridement is also effective in removing necrotic tissue [44]. Biosurgical debridement has been used in patients with large ulcers having significant necrotic material; that approach is limited by patient discomfort [45]. Recent Cochrane reviews have not shown sufficient evidence yet in favor a particular debridement method [46, 47]. However, we think that the choice of debridement methods is highly dependent on the etiology of the chronic wound.

Effect of Debridement on Wound Cells and Wound Environment in General

The aim of debridement is to provide enhanced efficacy of other therapies, be it growth factors, bioengineering skin, cell-based therapies, or other therapies. So, it is very important to characterize the effect of debridement on the whole wound bed, including the effect on the wound cells and wound microenvironment.

Some studies have reported that biosurgical (larva-induced) debridement procedure promotes diabetic foot wound healing by upregulating endothelial cell activity. However, the molecular pathways by which biosurgical debridement enhances angiogenesis are not clear [48]. Moreover, other studies have reported that biosurgical debridement modifies fibroblast adhesion and spreading across ECM protein surfaces, while keeping cells viable [49]. Remodeling of the WBP via ECM-degrading enzymes not only allows removing necrotic tissue by eliminating devitalized collagen but also stimulates cellular responses to injury, tissue remodeling, and wound healing. In this way, collagenase digestion of the ECM produces bioactive wound healing peptides which activate endothelial morphogenesis in vitro and might contribute to enhancing angiogenesis [50]. Moreover, it has been reported that after surgical debridement keratinocytes show normalized gene expression, which may allow increased ability to respond to growth factor topical applications [39].

In chronic wounds debridement must be done without injuring viable tissue and, thus, keeping healthy cells within the wound that are biologically capable of responding to therapies [39]. The use of a clinical method of wound bed scoring applicable in diabetic foot ulcers is recommended to know when one must stop debridement [51]. Moreover, there are other techniques to determine whether debridement was successful, including histological analysis, immunohistochemistry, or molecular markers [4]. Also, DNA microarrays or other gene profiling methods or signature may be used to identify gene expression and guide debridement of chronic wounds [39].

Other Options for Wound Bed Preparations (WBP)

There are basic and more advanced approaches for WBP to improve clinical outcomes in the treatment of diabetic ulcers, including (a) basic procedures (debridement, bacterial bur-

den control, and edema and exudate removal, hyperbaric oxygen therapy) and (b) advanced treatments (biological and tissue engineering therapies).

Wound infection is one of the most severe complications of diabetic foot ulcers and it often leads to amputation. Clinical infection in diabetic ulcers must be diagnosed and treated rapidly and adequately with debridement, systemic antibiotics, and topical antiseptics [52, 53]. However, the presences of biofilm, which shield bacteria from antibiotics and antiseptics, may render antimicrobial therapy less effective [17]. Debridement procedure and even ultrasound are being recommended in infected ulcers to avoid the establishment and spread of infection [54].

Dressings are helpful in diabetic ulcer treatment. Many dressings have been developed based on the hypothesis that reepithelization increases when wounds are kept moist. However, appropriate moist wound healing is difficult to achieve in diabetic ulcers because a delicate balance is needed to avoid maceration of tissues while promoting conditions that prevent eschar formation and facilitate cell migration within the wound [55]. Currently, there is no strong evidence for differences between wound dressings for any outcome in diabetic foot ulcers [56]. Edema control and removal of exudate are also critical in the management of diabetic ulcers. Edema removal reduces wound fluid, which has been shown to be harmful for the cells around the wound and may enhance bacterial colonization [5].

Pressure off-loading for avoiding constant trauma and is essential in diabetic wound healing. Although there are many types of pressure-relieving devices, total contact casts are considered by many to be the best standard method for off-loading and treating diabetic patients with neuropathic ulcers [57]. However, this is a common controversy.

Tissue hypoxia is a common issue in diabetic ulcers. Some studies suggest that hyperbaric oxygen therapy (HBOT), by exposure to 100% oxygen pressure of about 1 atmosphere, is strongly effective in reducing the rate of major amputations in patients with diabetic foot ulcers [58, 59].

Several growth factors have effects on cell regeneration, stimulation of proliferation, migration of keratinocytes, formation of granulation tissues, and promotion of fibroblast motility. Some studies support the use of human recombinant epidermal growth factor (rhEGF) in treating diabetic ulcers since it increases the rate of wound healing [60–64]. However, platelet-derived growth factor BB (PDGF-BB) is the only Food and Drug Administration (FDA)-approved topically applied recombinant growth factor in the USA for the treatment of diabetic foot ulcers [65, 66]. An appropriate WBP of chronic wounds before the application of the growth factor might be useful to improve the efficacy of these therapies with growth factors. In fact, there is evidence that extensive debridement of diabetic ulcers seems to be synergistic with the application of PDGF-BB [38]. It should be noted that the effectiveness of PDGF-BB in diabetic neuropathic foot ulcers is marginal.

It has been reported that platelets release multiple functional growth factors and cytokines (including PDGF, TGF-β1, vascular endothelium growth factor (VEGF), epithelial growth factor (EGF), *Basic fibroblast growth factor* (bFGF), brain-derived neurotrophic factor, and hepatocyte growth factor) [67]. Some studies have suggested that plasma rich in platelets (PRP) may improve diabetic foot ulcers [68]. Figure 16.3 shows our experience in the acceleration of wound closure in diabetic wounds by topical delivery of PRP. However, a 2016 Cochrane systematic review on the topical application of PRP found no conclusive evidence for its effectiveness in treating foot ulcers [69].

Several bioengineered skin substitutes have become available for the treatment of acute and chronic wounds. These constructs are a combination of complex matrix products and cells, others are acellular [70]. A 2016 Cochrane systematic review provides evidence that some skin substitutes can augment standard care to increase complete ulcer closure in diabetic foot ulcers. However, long-term studies are needed to support the effectiveness of these therapeutic approaches [71].

Different stem cell therapies have been identified as advanced therapies for treating nonhealing wounds. Therapy with mesenchymal stem cells (MSC) for promoting wound healing is interesting due to their differentiating potential, their immunomodulating properties, and their paracrine effects [72]. MSC-based therapy has emerged as a promising therapeutic strategy for treating chronic wounds, such as diabetic ulcers [23, 73]. Gallagher and colleagues reported that bone marrow-derived endothelial progenitor cells (EPCs) play a relevant role in the process of neovascularization in response to ischemic conditions, which is the case in diabetic wounds [74]. Moreover, some studies have shown the potential of cellular reprogramming to generate human induced pluripotent stem cells (iPSCs) as a source of autologous cell-based therapies. Gerami-Naini and colleagues demonstrate that fibroblasts derived from diabetic ulcers can be efficiently reprogrammed to iPSCs and differentiated into fibroblasts. This might lay the groundwork for using these cells as a therapeutic approach to correct wound cellular repair defects [75]. However, a better understanding of the phenotypic changes in resident cells and the identification of the diabetic ulcer genome and correlation of transcriptional profiles with clinical outcomes would allow determining specific genes that prevent a wound from healing and should further be useful to improve the therapeutic outcomes of biological therapies.

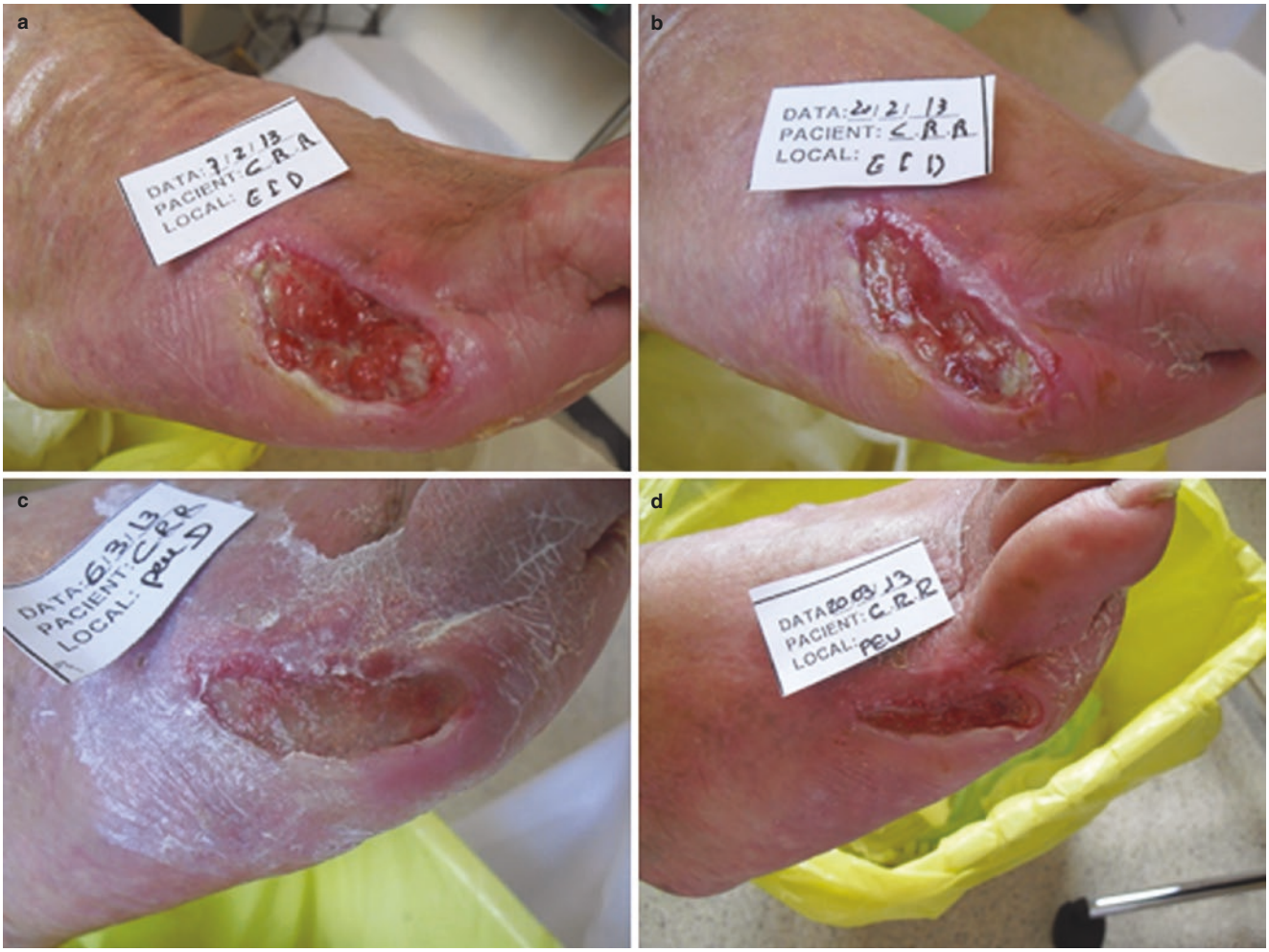

Fig. 16.3 Diabetic wound treated by topical application of plasma rich in platelets (PRP). Diabetic wound progression along several sessions of biological therapy with PRP (**a**–**d**)

Reappraisal of Wound Bed Preparation

Some of our recent new findings around the altered phenotype of wound cells and also the capacity of these cells to resist pressure have let us to reconsider that a reappraisal of WBP is needed.

For many years, investigators have accepted the hypothesis that wound bed resident cells from chronic wounds are either senescent or unable to respond to growth factors and other stimulatory activities and mediators. However, our ongoing experiments in dermal fibroblasts show that this hypothesis may not be correct. Our data are showing that chronic wound fibroblasts (as a typical marker of resident wound cells) may not be "dormant" after all and may actually be excessively active in their proliferative and migratory activities [64]. These results are in agreement with recent results showing that diabetic foot ulcer-derived fibroblasts are also active in a three-dimensional in vitro model and also in an in vivo animal model of chronic wound healing [76]. Our findings suggest that the ulcer microenvironment, more than the wound cells phenotype, could affect wound bed cellular activity. So, more studies about how WBP procedures modify the biological factors of wound bed are needed.

The TIME concept and WBP approach need a reappraisal for additional reasons. TIME, specifically, did not evolve enough scientifically to advance the field. That would have required well-planned experiments and clinical research. Instead, TIME may have become a rather stagnant concept. An example of that is quite useful. Thus, several dressings increase the pressure on the wound edge by fixing the tissue in place or by impairing the redistribution of pressure, especially in plantar foot ulcers. Also, some studies are needed to determine whether pressure could be another wound microenvironment factor that could affect wound cells functionality (migration and proliferation).

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Part III

Management of the Diabetic Foot

Microbiology and Treatment of Diabetic Foot Infection

17

Mary T. LaSalvia and Adolf W. Karchmer

Abstract

The foot of patients with diabetes mellitus is affected by several processes which not only contribute to the development and progression of infection but on occasion alter the appearance of the foot in ways that may obscure the clinical features of local infection. Neuropathy involving the motor fibers supplying muscles of the foot causes asymmetric muscle strength, which in turn results in foot deformities and maldistribution of weight (or pressure) on the foot surface. Dysfunction of the sensory fibers supplying the skin and deeper structural elements of the foot allows minor and major injury to these tissues to proceed without appreciation by the patient. As a result of neuropathy, the foot may be dramatically deformed, ulcerate in areas of unperceived trauma (mal perforans), and on occasion be warm and hyperemic in response to deep structural injury (acute Charcot's disease). This warmth and hyperemia may be misinterpreted as cellulitis and an ulceration, while a major portal of entry for infection, may be uninfected. In the patient with diabetes, peripheral neuropathy may develop in isolation or commonly in parallel with atherosclerotic peripheral vascular disease. The latter involves major inflow vessels to the lower extremity but commonly is associated with occlusive lesions of the tibial and peroneal arteries between the knee and ankle. The resulting arterial insufficiency can alter the appearance of the foot and obscure infection. Rubor may reflect vascular insufficiency rather than inflammation and conversely pallor may mute the erythema of acute infection. Gangrene and necrosis may be primarily ischemic or may reflect accelerated ischemia in the setting of infection. In sum, the diagnosis of infection involving the foot in patients with diabetes requires a careful detailed examination of the lower extremity and its blood supply.

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The Diagnosis of Foot Infections

The initial step in the diagnosis of a foot infection in a patient with diabetes is to recognize those patients at greatest risk and to maintain a suspicion for infection. Foot infections often present with more subtle findings in patients with diabetes because of impaired leukocyte function, ischemia, and peripheral neuropathy; thus, clinicians should evaluate any foot wound for the possibility of infection [1, 2]. Suspicion for infection should be heightened if additional clinical factors that have been significantly associated with foot infection are present. These include peripheral arterial disease with absent pulses or an ankle brachial index of <0.9, loss of protective sensation, a history of recurrent foot ulcers or prior amputation, foot ulcers of >30 days duration, a wound that extends to bone, i.e., a positive probe-to-bone test (see Osteomyelitis), and a traumatic wound [3, 4]. Thereafter, infection is diagnosed clinically and to varying degrees supported by test results. Finding purulent drainage (pus) or two or more signs or symptoms of inflammation (erythema, induration, swelling, pain, tenderness, or warmth) is indicative of infection. Clinical signs on occasion belie the significance and severity of infection. A minimally inflamed but deep ulceration may be associated with underlying osteomyelitis [5]. Serious limb-threatening infection may not result in systemic toxicity. For example, among patients hospitalized for limb-threatening infection only 12–35% have significant fever [6–8]. In fact, fever in excess of 102 °F suggests infection involving deeper spaces in the foot with tissue necrosis and undrained pus, extensive cellulitis, or bacteremia with the potential for hematogenous seeding of remote sites. Laboratory studies may be supportive of the diagnosis of these infections but must be interpreted in the context of clinical findings. The erythrocyte sedimentation rate and C-reactive protein concentration may be normal in infected patients, and in up to 50% of patients with deep foot infection the white blood cell count may be normal [9, 10]. Elevated concentration of C-reactive protein and procalcitonin can help distinguish mild or moderately infected ulcers

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from those that are uninfected [11]. In addition to the presence of classic pathogens, foot ulcers are often contaminated or colonized by commensal organisms that on occasion become pathogens. As a consequence, cultures, while essential in the assessment of the microbiology of foot infections, do not in isolation establish the presence of infection. Unless the cultured material is obtained from deep tissue planes by percutaneous aspiration, the results of cultures must be interpreted in the clinical context.

The Diagnosis of Osteomyelitis

The evaluation of the wound should also focus on the presence of possible bone involvement. The diagnosis of osteomyelitis is often difficult because of confounding Charcot neuro-osteoarthropathy and adjacent soft tissue infection. In the diabetic foot, osteomyelitis almost always results from direct extension through an overlying infected chronic ulcer. Clinical features that increase the probability of osteomyelitis are an ulcer larger than 2 cm^2, an ulcer extending down to bone, and an ESR of greater than 70 mm/h [12]. The depth of an ulcer should be explored by gentle probing of the ulcer base with a sterile, blunt metallic probe. The probe-to-bone (PTB) test, which is performed prior to extensive debridement, can identify bone that is exposed but not visible on examination of the base of a pedal ulcer [1, 13]. Probing is generally tolerated without pain due to the nearly universal presence of marked sensory neuropathy. The positive and negative predictive value of the PTB test is dependent on the prevalence of osteomyelitis in the population studied. When performed on moderate or severely infected foot ulcers, a positive PTB test is highly suggestive of osteomyelitis; however, a negative test does not exclude the diagnosis. In an uninfected wound, a positive PTB is not specific for osteomyelitis but the diagnosis is made less likely with a negative test result. In a prospective study of 75 patients with 76 clinically infected foot ulcers, palpating bone on probing the pedal ulcer had a sensitivity of 66%, a specificity of 85%, a positive predictive value (PPV) of 89%, and a negative predictive value (NPV) of 56% for diagnosing osteomyelitis [14]. A prospective study including 210 foot lesions evaluated clinical signs of infection, radiographic signs, ulcer culture, and the probe-to-bone test. The probe-to-bone test was of greatest diagnostic value with a sensitivity of 94%, a specificity of 78%, a PPV of 95%, and a NPV of 91% [15]. A recent systematic review of the accuracy of the PTB test to diagnose diabetic foot OM demonstrated a pooled sensitivity of 87% and specificity of 83%, further supporting the ability of the PTB test to accurately diagnose osteomyelitis in the diabetic foot [16].

Plain radiographs of the foot are a reasonable first imaging study to assess for osteolytic bone changes and periosteal elevation, suggestive of osteomyelitis. The combined use of serial PTB test and plain radiography has been found to increase agreement among clinicians of the diagnosis of osteomyelitis [17]. The low sensitivity of plain radiography early in infection frequently leads to consideration for alternative modes of imaging such of technetium-99 bone scan and MRI. MRI has become the study of choice when further imaging is required due to its enhanced sensitivity [1, 13]. The use of imaging in the diagnosis of osteomyelitis is reviewed in detail in Chap. 5 (radiographic changes of the diabetic foot).

Bone infection can be confirmed by the histopathologic findings of osteomyelitis. Bone biopsy may be falsely negative because of either patchy infection or reduced culture yield in the setting of prior antibiotics [18]. In a study of the diagnosis of osteomyelitis, bone culture obtained from surgical debridement was compared to bone histopathology on 44 bone specimens, the two tests performed similarly [19]. If surgical debridement is not undertaken, percutaneous biopsies have been shown to be safe and superior to superficial swabs for detecting organisms causing osteomyelitis [20]. In a retrospective study of 76 patients with pathology confirmed osteomyelitis, bone and swab cultures were fully concordant in only 17% of patients, and bacteria present on bone culture were isolated from the corresponding swab in only 30% of patients [20].

The Severity of Foot Infections

Clinicians should routinely utilize a validated classification system when assessing the severity of diabetic foot infection [1]. Multiple classification schema have been designed to define the severity of foot wounds with or without infection in patients with diabetes. Some such as the widely used Wagner system include infection only in one grade [21]. Others, focused on subtle grading of features of infection, require a scoring sheet and are thus too complex for routine clinical use. Well-studied systems to classify the severity of infection have been developed by the Infectious Diseases Society of America (IDSA) and the International Working Group on the Diabetic Foot (IWGDF) [1, 2, 22, 23]. The IWGDF utilizes the acronym PEDIS to classify diabetic foot wounds; PEDIS stands for PErfusion, Depth, Infection and Sensation [23]. The IDSA and IWGDF schema are nearly identical and classify wounds from having no infection to being severely infected (Table 17.1). Infected wounds are then subdivided into mild, moderate, and severe infection by using the depth of a wound, presence of ischemia, presence and extent of infection, and presence of systemic toxicity [24]. Increased severity in the IDSA classification schema, e.g., moderate and severe infection, correlates with the need for hospitalization and amputation [22].

Table 17.1 Classification of severity of diabetic foot infection

Clinical manifestation of infection	Infection severity[a]
Wound lacking purulence of any manifestations of inflammation	Uninfected
Presence of ≥2 manifestations of inflammation (purulence, or erythema, pain, tenderness, warmth, or induration), but any cellulitis/erythema extends ≤2 cm around the ulcer, and infection is limited to the skin or superficial subcutaneous tissues; no other local complications or systemic illness	Mild
Infection (as above) in a patient who is systemically well and metabolically stable but which has ≥1 of the following characteristics: cellulitis extending >2 cm, lymphangitic streaking, spread beneath the superficial fascia, deep-tissue abscess, gangrene, and involvement of muscle, tendon, joint or bone	Moderate
Infection in a patient with systemic toxicity or metabolic instability (e.g., fever, chills, tachycardia, hypotension, confusion, vomiting, leukocytosis, acidosis, severe hyperglycemia, or azotemia)	Severe

Adapted from reference [24] with permission
[a]In the setting of severe ischemia all infections are considered severe

In addition to assessing the wound, the affected limb and foot should be assessed for signs of arterial ischemia, venous insufficiency, neuropathy, or biomechanical factors which promote infection [1]. Systemic signs and symptoms of infection include fever, chills, alteration in mental status, hemodynamic instability, and metabolic derangements such as hyperglycemia, acidosis, or renal failure. Of note, hyperglycemia occurs almost universally in patients with non-limb-threatening and limb-threatening infection. Fever is found primarily in patients with extensive cellulitis and lymphangitis, infection (abscesses) loculated in the deep spaces of the foot, bacteremia, or hematogenously seeded remote sites of infection [6–8].

After adjusting for prior medical therapy and antibiotic exposure which is likely to result in resistant organisms, these classification schemes allows one to anticipate the organisms causing wound infection and thus is an excellent point of departure from which to plan antimicrobial therapy.

Microbiology

Cultures of open foot ulcers cannot be used to establish the presence of infection. Foot ulcers whether infected or not will often contain multiple commensal or colonizing bacteria, some of which have the potential to become invasive pathogens. As a foot ulcer transitions from uninfected to infected, organisms isolated from the ulcer cavity include both colonizing flora and invasive pathogens. Assigning specific significance to organisms isolated from ulcers may be difficult. Sapico and colleagues demonstrated that the organism cultured from specimens obtained by aspiration or by curettage of the base of a cleansed ulcer were most concordant with those isolated from necrotic infected tissue excised from adjacent to the ulcer base [25]. Of note, cultures of aspirated material failed to yield pathogens recovered from curettage or excised tissue in 20% of patients. Although not endorsed strongly by the IDSA guidelines or other experts, culture of material obtained on swabs of the deep ulcer base may provide useful information. Slater et al. found that in wounds that did not extend to bone essentially the same organisms were recovered from cultures of swab specimens and deep tissue specimens. When wounds extended to bone, cultures of swab specimens recovered only 65% of organisms cultured from deep tissues [26]. Examining initial wound cultures, Pellizzer et al. also found that culture of swab specimens taken from deep in the ulcer yielded the same bacterial species as did cultures of deep tissue biopsies, with the exception that *Corynebacterium* species, likely colonizers or contaminants, were isolated from swab cultures [27]. When surgical or aspiration specimens are not readily available for culture, antibiotic therapy can be designed with reasonable confidence based upon the culture results from specimens obtained by curettage of the ulcer base. Accordingly, culture of material obtained from an ulcer base by curettage after the ulcer has been cleansed and debrided is recommended. Culture of material swabbed from an ulcer base is a less desirable alternative. In contrast, cultures obtained from ulcers are not adequate for the design of antimicrobial therapy for osteomyelitis when the infected bone is to be debrided piecemeal, as opposed to resected en bloc. In this situation, more precise biopsy-based culture information is highly desirable [24, 28].

It is possible to sense the major pathogens causing non-limb-threatening and limb-threatening foot infections from the microbiology in clinical reports, even though most specimens are obtained through the ulceration. Doing so requires interpretation to adjust for the inclusion of organisms of known low invasive potential and likely to be commensals or colonizers. In non-limb-threatening infections, particularly those occurring in patients who have not previously received antimicrobial therapy, *Staphylococcus aureus* and streptococci, particularly group B streptococci, are the predominant pathogens [24, 28–32]. *S. aureus* has been isolated from more than 50% of these patients, and in more than 30% *S. aureus* is the only bacteria isolated [29]. Although there are differences between geographic areas, *S. aureus* causing infections in the feet of diabetics are, as in other skin and soft tissue infections, increasingly methicillin-resistant (MRSA). In one study the prevalence of MRSA increased

from 11.6 to 21.9% from 2003 to 2007 [33]. Other studies of diabetic foot infections have also noted an increase in the percent of *S. aureus* that are methicillin-resistant [34–36]. Risk factors for MRSA diabetic foot infection include prolonged wound duration, inpatient management, and chronic kidney disease [36, 37].

Limb-threatening foot infections, which often involve deeper tissues and are typically chronic, as well as previously treated, are commonly polymicrobial. Cultures from these infections yield on average 2.3 to 5.8 bacterial species per culture. Both gram-positive cocci and gram-negative rods are commonly isolated from a single lesion, and in 40% of infections both aerobic and anaerobic organisms are recovered [6, 24, 25, 28, 30, 32, 38–40] (Table 17.2). Individual cultures have yielded on average 2.9–3.5 aerobes and 1.2–2.6 anaerobes [41]. *S. aureus* (including methicillin-sensitive and methicillin-resistant isolates), streptococci (particularly group B streptococci), and facultative gram-negative bacilli (*Proteus* species, *Enterobacter* species, *Escherichia coli*, *Klebsiella* species) and *Pseudomonas aeruginosa* are the predominant pathogens in these infections. Among the anaerobes, *Peptostreptococcus* species, *Prevotella* species, and *Bacteroides* species, including those of the *B. fragilis* group, are recovered frequently [41, 42]. Of note, *Clostridium* species are recovered infrequently. Although anaerobes are recovered from 41 to 53% of limb-threatening infections in clinical trials, with optimal methods these organisms can be recovered from 74 to 95% of these infections [41]. The frequency of isolating anaerobic bacteria is greatest in those patients with the most severe infections, particularly those where infection involves necrotic gangrenous tissue and amputation is often required. Fetid infections suggest infection with anaerobes; however, anaerobes including *B. fragilis* may be recovered from infections that are not particularly foul smelling. Hence, clinical clues beyond the major categorization of infections as non-limb-threatening or limb-threatening are not sufficient to predict the microbiology of foot infections.

The spectrum of bacterial species recovered from foot infections, especially those that are limb-threatening, can be dramatically altered by prior failed antimicrobial therapy or contact with the health care system. While *Pseudomonas aeruginosa*, *Acinetobacter* species, *Enterobacter* species, and other antibiotic-resistant gram-negative bacilli are uncommon in previously untreated infections, these organisms are not infrequent isolates from infected chronic ulcers [6, 37, 40] and from ulcers in patients with previous hospitalizations for the same wound [43, 44]. Similarly, MRSA may be encountered commonly in patients with chronically infected foot ulcers that have persisted in spite of multiple prior courses of antimicrobial therapy or in patients with extensive health care requirements, e.g., chronic dialysis,

Table 17.2 Microbiology of moderate or severe limb-threatening infections in patients with diabetes[a]

	Percent of patients (Number patients)						
Organisms	Gibbons et al. (42)	Hughes et al. (50)	Bamberger et al. (51)	Scher et al. (65)	Grayson et al. (96)	Citron et al. (427)	Gadepalli et al. (80)
Aerobic							
S. aureus	22	25	22	23	54	15	14
S. epidermidis	12	14	19	18	12	11	8
Enterococcus spp.	16	17			28	12	11
Streptococcus spp.	13	20	41	54	55	10	
Corynebacterium spp.	7		8			7	
E. coli	7	3	1	19	6	1	12
Klebsiella spp.	4	7	4	10	5	2	7
Proteus mirabilis	11	11	5	36	9	2	13
Enterobacter spp.	3	7	7		9	2	1
Other -							
Enterobacteriaceae	2	5	7	50	17	2	10
P. aeruginosa	3	0	5	15	8	2	9
Acinetobacter spp.	1	0	0		7	1	
Anaerobic							
Gram-positive cocci	21	40	14	52	12	13	7
Bacteroides fragilis		5	4			3	7
Bacteroides melaninogenicus		11				4	
Other -							
Bacteroides spp.	6	2	5	55	30	3	
Clostridium spp.	2	1	3	23		1	1
Other anaerobes		13	2	20	14	6	3
Number isolates/infection	2.76	3.62	2.88	5.76	2.77	3.8	2.3

[a]Specimens obtained by various routes, including deep ulcer swabs, curettage of the ulcer base, aspiration or tissue biopsy
Data from references: [6, 38–40, 71, 81, 95]

hospitalization for comorbid conditions, residence in skilled nursing facilities, or particularly those with a prior history of infection with this organism [45]. These resistant bacteria are probably acquired nosocomially or alternatively emerge from endogenous flora during repetitive antibiotic treatment of nonhealing foot ulcers. Accordingly, when selecting an antimicrobial regimen to treat a foot infection in a patient who has had contact with the health care system or prior courses of antibiotics, physicians should anticipate the presence of antibiotic-resistant pathogens.

It is also important to note the potential geographic variation in antimicrobial resistance rates in diabetic foot infection. A recent prospective multicenter study in Turkey collected 522 specimens from infected diabetic foot wounds for culture from 447 individual patients [46]. Gram-negative organisms constituted 60.2% of all isolates, the most common of which was *E. coli* (15%) followed by *P. aeruginosa* (12.4%). Antimicrobial resistance for these isolates was higher in specimens taken from patients with moderate-severe infection compared to mild-moderate infection. While *S. aureus* was the most common gram-positive organism isolated (11.4%), MRSA was present in only 1.8% of cultures. In an additional study performed in India in 2014, 51 isolates were obtained from 50 patients with diabetic foot infection, of which 21 (41%) isolates were *Staphylococcus aureus*, 4 (19%) of which were MRSA. Gram-negative bacilli accounted for 51% of the isolates, of which 44% were ESBL producers [47]. Given notable geographic variation, the expected microbiology of diabetic foot infection needs to be based on the local prevalence of pathogens, especially antibiotic-resistant strains, but amplified by culture results.

The role in infection of relatively avirulent bacteria, many of which are part of skin flora, is uncertain. *Staphylococcus epidermidis* and other coagulase-negative staphylococci have been recovered, usually in conjunction with other bacteria, from 15 to 35% of foot infections and may reflect ulcer colonization. On the other hand, *S. epidermidis* on occasion has been the sole organism recovered from deep tissue curetted from an infected ulcer; this suggests that these organisms may be pathogens in some patients. Enterococci, viridans streptococci, and *Corynebacterium* species, organisms that are often considered colonizers and not pathogens when isolated from skin and soft tissue infections, are among the isolates recovered frequently from polymicrobial limb-threatening foot infections. When recovered from specimens in conjunction with typical pathogens, these organisms are often disregarded [24, 28]. Often, foot infections respond to therapy with antimicrobials which are active in vitro against the pathogens but not against these presumed colonizers [41, 48]. These observations support the designation of these organisms as non-pathogens; alternatively, they could indicate that with the eradication of major pathogens, host defenses and surgical debridement can control these less virulent organisms. On occasion enterococci, viridans streptococci, and *Corynebacterium* species are isolated from uncontaminated specimens and may even be the sole bacterial isolate from an infection [30]. Thus, these organisms too should not be routinely disregarded but rather interpreted in the clinical context.

Microbiologic Assessment

Clinically uninfected ulcers should not be cultured. When infection is present, a microbiologic diagnosis will usually facilitate subsequent therapy, particularly in the setting of limb-threatening infection or that occurring after failure of prior antimicrobial therapy [7, 31, 49]. While cultures of tissue obtained aseptically at surgery or purulent specimens aspirated percutaneously are more likely to contain only true pathogens, obtaining these specimens before initiating therapy is often either impractical or not feasible (no abscess present). Accordingly, before beginning antibiotic therapy the skin should be cleansed and any overlying eschar debrided. Then specimens for culture should be obtained by curettage of the necrotic base of the ulcer. Specimens should be handled and processed as both routine wound cultures and primary anaerobic cultures. As noted, specimens obtained by swabbing deep in the ulcer or from curetted tissue in the base of the ulcer may provide a reasonable assessment of infecting organisms [26, 27, 50]. If patients have been febrile, blood cultures should also be obtained before initiating antimicrobial therapy. With subsequent debridement during early days of therapy, specimens from necrotic purulent tissue or exposed bone should be cultured again. Concurrent antimicrobial therapy may preclude isolation of susceptible organisms during effective therapy; however, resistant organisms missed on the initial cultures can be recovered from these later debridement specimens [27]. Treatment of osteomyelitis involving bones in the forefoot that will be totally resected does not require specific bone cultures; that is, antibiotic therapy can be designed using the results of appropriate wound cultures. If en bloc resection of the involved bone, i.e., foot-sparing amputation, is not performed, more precise microbiologic data from bone biopsy would be desirable to allow selection of optimal antibiotic for therapy [18, 24, 28]. Biopsy of abnormal bone underlying infected ulcers is generally safe and in severely neuropathic patients may not require anesthesia. Bone in the mid-foot or hindfoot that can be probed or that lies beneath an ulcer and appears infected on imaging studies should be biopsied for culture and histopathology. Ideally this should be done either surgically or percutaneously through a route other than the ulcer with fluoroscopic guidance [18, 24, 28]. Here where debridement is likely to be piecemeal, rather than en bloc resection of all involved bone, precise microbiologic data

from bone is required so that optimal antimicrobial therapy can be selected. Alternatively, bone biopsy may be deferred when bone that remains unexposed after debridement and wherein osteomyelitis is not strongly suspected based on radiologic findings; here the infection can be treated as if it is limited to soft tissue. Careful clinical and radiologic follow-up of this bone in 2–4 weeks will often resolve the question of osteomyelitis.

Treatment

Debridement and Surgery

With the exception of cellulitis or lymphangitis arising from an unrecognized (or microscopic) portal of entry, moderate or severely infected foot ulcers generally require debridement. Debridement should be done surgically rather than by chemical or enzymatic agents [51]. Urgent surgical intervention is required when patients present with foot infection complicated by extensive necrosis or gangrene, crepitus or gas in tissues on imaging, necrotizing fasciitis (or pain out of proportion to findings thus suspected necrotizing fasciitis), critical ischemia, or life-threatening sepsis. For apparent moderate non-limb-threatening infections, debridement may be limited but nevertheless allows full evaluation of the portal of entry and prepares the site for culture. Occasionally, what appeared to be a non-limb-threatening infection is discovered on debridement to actually be more severe with extension of infection to deep tissue planes. Severe limb-threatening infection by virtue of extension to deep tissue planes requires surgical debridement [7, 49]. Early surgical intervention can reduce the duration of hospitalization and the need for major amputations [52]. Failure to decompress involved compartments and debride necrotic tissue and drain purulent collections increases the risk of amputation [7, 49, 52, 53]. Percutaneously placed drains or aspiration drainage is inadequate; rather devitalized tissue must be resected and purulent collections drained by incision. Uncertainty about the patient's arterial circulation status should not delay initial debridement but should prompt an evaluation of arterial supply and a vascular surgery consultation. Effective debridement may require multiple procedures as the extent of tissue destruction becomes progressively more apparent. Optimal surgical treatment, that which minimizes tissue loss and results in a suitable weight-bearing foot, requires a thorough understanding of resulting foot function, avoidance of subsequent deformities that will predispose to recurrent ulceration, and recognition of the potential need for revascularization to insure healing [53]. The experience of the surgeon in this area and the availability of vascular surgery support are important in achieving optimal results [53]. If the infection has destroyed the function of the foot or if it threatens the patient's life, a guillotine amputation to allow prompt control of the infection with a subsequent definitive closure is advised [54].

Antibiotic Therapy

The potential therapeutic or prophylactic benefits of systemic antibiotic therapy in patients with uninfected neuropathic ulcers is a subject of debate. One controlled trial showed no benefit from antibiotic therapy [55]. In view of the potential adverse consequences, including colonization with resistant bacteria, antibiotic therapy is not recommended for clinically uninfected neuropathic ulcers [24, 51]. Similarly, continuation of antibiotics beyond a limited course that was sufficient to eradicate infection has not been required to accomplish the healing of ulcers that remain open [24, 29, 56].

Topical antimicrobials, including silver sulfadiazine, polymixin, gentamicin, and mupirocin, have been used to treat selected soft tissue infections; however, this approach has not been studied in foot infections. A cationic peptide antimicrobial, pexiganin acetate, used as a 1% cream applied topically, was nearly as effective as oral ofloxacin in treating mildly infected foot ulcers [57]. Although antimicrobials have been applied topically to foot infections, it seems unlikely that the topical route would result in effective tissue concentrations of the antimicrobial. In addition, the expense of these dressings must be considered as well as their potential for causing local adverse effects and the potential for selecting antimicrobial-resistant organisms. Accordingly, current guidelines do not advocate for the use of topical antimicrobials for most clinically uninfected wounds, nor the use of silver-based dressings for clinically infected wounds [1].

After obtaining appropriate cultures, antimicrobial treatment should be started promptly for all clinically infected ulcers. Therapy of foot infections in patients with diabetes is begun empirically and thereafter revised. Revision should be based upon the results of cultures, which were obtained prior to therapy and on occasion during therapy, plus the clinical response of the infection empiric therapy. Knowledge of the spectrum of bacteria which cause non-limb-threatening infection and limb-threatening infection, as well as the changes in these organisms that might have been induced by selected circumstances, e.g., prior antimicrobial treatment, serves as the basis for selecting effective empiric therapy. The potential toxicity of various antibiotics for individual patients and the unique vulnerability of patients with diabetes as a group must be considered. The route of therapy should be based on the severity of infection. Oral therapy is sufficient for most mild infections as well as many moderate infections when an antibiotic with good oral bioavailability may be utilized. Antibiotic therapy is administered intravenously when patients are systemically ill, have severe local

infection, are unable to tolerate oral therapy, or are infected by bacteria that are not susceptible to available oral antimicrobials. Some antimicrobials are fully bioavailable after oral administration, e.g., selected fluoroquinolones, clindamycin, and metronidazole, trimethoprim/sulfamethoxazole, and linezolid. When appropriate microbiologically and clinically, these could often be used in lieu of initial parenteral therapy. After control of infection, continued therapy commonly can be effected with oral agents contingent upon the susceptibility of the implicated bacteria. For patients who require prolonged courses of parenteral therapy, e.g., for osteomyelitis, generally treatment can be provided in an outpatient setting [58].

Empiric therapy for patients with mild or moderate infection, many of whom can be treated as outpatients, is directed primarily at aerobic gram-positive cocci, i.e., staphylococci and streptococci (Table 17.3) [24, 28, 32, 51]. At a time when MRSA were uncommonly encountered, Lipsky et al. demonstrated that oral therapy with clindamycin or cephalexin for 2 weeks in patients with previously untreated non-limb-threatening foot infection resulted in satisfactory clinical outcome in 96% and 86%, respectively [29]. Caputo et al. in a retrospective study reported that 54 of 55 patients with non-limb-threatening infections were improved or cured with oral therapy, primarily first generation cephalosporins or dicloxacillin, directed at staphylococci and streptococci [31]. Currently when selecting therapy for mild or moderate infection, the need for MRSA coverage must also be considered. Treatment that encompasses MRSA is required if there is a known history of MRSA colonization or if local prevalence is high. Oral trimethoprim-sulfamethoxazole or linezolid may be used for this purpose. Trimethoprim-sulfamethoxazole may be less effective against streptococci and thus should be combined with a second agent. Use of doxycycline or clindamycin, both of which may be active against some MRSA, should be based on local susceptibility patterns. If MRSA is not suspected, oral agents directed at MSSA and streptococci such as cephalexin, dicloxacillin, amoxicillin-clavulanic acid, levofloxacin, moxifloxacin, and clindamycin may be considered.

Table 17.3 Selected antibiotic regimens for initial empiric therapy of mild non-limb-threatening foot infections in patients with diabetes mellitus

Antimicrobial regimen[a]
Cephalexin 500 mg p.o. q 6 h
Clindamycin 300 mg p.o. q 8 h
Amoxicillin-clavulanate (875/125 mg) one q 12 h
Dicloxacillin 500 mg p.o. q 6 h
Levofloxacin 500–750 mg p.o. q d
Moxifloxacin 400 mg p.o. q d
Trimethoprim/sulfamethoxazole DS, one or two tablets p.o. bid[b]
Linezolid 600 mg p.o. bid[b]
Doxycycline 100 mg po bid

[a]Doses for patients with normal renal function

[b]Use if clinical information suggests possible methicillin-resistant *S. aureus* infection (MRSA). Trimethoprim/sulfamethoxazole may be less effective against streptococcal infection and require addition of second antimicrobial. Clindamycin and doxycycline are active against some MRSA

If patients with superficial ulcers present with more extensive cellulitis, that warrants hospitalization and parenteral antimicrobial treatment, intravenous therapy should be initiated. Cefazolin should be effective in patients without MRSA risk factors. Linezolid, which is fully bioavailable when administered by mouth and thus can be given orally or intravenously, is generally active against MRSA and thus could be used as anti-staphylococcal therapy for non-limb-threatening or limb-threatening foot infections [59]. Other antimicrobials active against MRSA available for intravenous administration in the setting of more extensive cellulitis or severe foot infections include vancomycin, daptomycin, telavancin, and ceftaroline [59–66]. The duration of treatment, which in the final analysis is determined by the time course of the clinical response, is usually 1–2 weeks.

For patients with severe infection, the randomized controlled trials to date have not clearly delineated a preferred single agent or combination of agents [67, 68]. In selecting empiric therapy for severe limb-threatening foot infections, reasonable principles emerge from clinical trials and other published studies [7, 24, 28, 32, 49, 51]. The choice of agents used empirically should be based upon the known polymicrobial nature of these infections with modification, where appropriate, to address anticipated highly resistant pathogens that might have been selected in the process of prior hospitalizations and treatment (Table 17.4) [69]. In addition, drug selection should attempt to minimize toxicity and be cost effective. Given the high prevalence of MRSA, either hospital acquired or the so-called community acquired variant, which has become commonplace, empiric therapy for limb-threatening infection should include an agent effective against MRSA. These agents will also provide therapy for infections caused by streptococci, including Group B organisms. Additionally, when infection occurs in a chronic ulcer which has failed to heal despite treatment with multiple antibiotics, empiric therapy should be effective against an array of Enterobacteriaceae including potentially multidrug-resistant organisms. Anaerobes, including *B. fragilis*, should be treated empirically in the more severe infection where there is tissue necrosis and gangrene or the wound is fetid. In limb-threatening infection (but not in life-threatening infection) initial empiric therapy does not have to be effective in vitro for all potential pathogens. Broad spectrum therapy which is active against many, but not necessarily all, gram-negative bacilli, as well as against anaerobes, *S. aureus* and streptococci when combined with appropriate debridement and good wound care may be as effective as even broader spectrum antimicrobial therapy. Adequate debridement not

Table 17.4 Antibiotics for empiric therapy of moderate or severe limb-threatening foot infection[a]

Antibiotic agent	Comments
Vancomycin	Active against streptococci, staphylococci including MRSA
Daptomycin	Active against streptococci, staphylococci including MRSA
Linezolid	Active against streptococci, staphylococci including MRSA
Telavancin	Active against streptococci, staphylococci including MRSA
Ceftaroline	Active against streptococci, staphylococci including MRSA and many Enterobacteriaceae (not ESBL producers, *P. aeruginosa)*
Levofloxacin	Active against streptococci and staphylococci (not MRSA) and many gram-negative bacilli
Moxifloxacin	Active against streptococci and staphylococci (not MRSA) and many gram-negative bacilli
Amoxicillin-clavulanate	Active against streptococci and staphylococci (not MRSA) and many gram-negative bacilli (not *P. aeruginosa*), also active against anaerobes
Piperacillin-Tazobactam	Active against streptococci and staphylococci (not MRSA) and many gram-negative bacilli including *P. aeruginosa*, also active against anaerobes
Imipenem-Cilastatin	Active against streptococci and staphylococci (not MRSA) and many gram-negative bacilli including *P. aeruginosa*, also active against anaerobes. Use when considering ESBL producing organisms
Ertapenem	Active against streptococci, staphylococci (not MRSA), many gram-negative bacilli including ESBL producers (not *P. aeruginosa*), active against anaerobes. Use when considering ESBL producing organisms
Ceftriaxone	Active against streptococci, staphylococci, and many gram-negative bacilli (not ESBL producers, *P. aeruginosa*, or anaerobes)
Cefepime/ceftazidime	Active against many gram-negative bacilli and *P. aeruginosa* (not against ESBL producers)
Metronidazole	Only active against anaerobes

[a]Often may need combined therapy, especially when considering MRSA and gram-negative bacillus polymicrobial infection
ESBL Extended spectrum beta-lactamase (use imipenem-cilastatin or ertapenem). Use doses suggested for complicated skin-soft tissue infection unless concomitant infection requires higher dose. Not all agents are approved by U.S. Food and Drug Administration (FDA) for treatment of diabetic foot infections

only shortens required duration of therapy but is required for effective therapy. However, patients with life-threatening infections, e.g., those with hypotension or severe ketoacidosis, should be treated with maximal broad spectrum regimens. These might include a carbapenem and an agent directed against MRSA plus, if highly resistant gram-negative bacilli are anticipated, gentamicin or another aminoglycoside can be added. In these patients, emergent debridement is essential for satisfactory outcome.

Multiple antibiotics have been demonstrated to be effective therapy in prospective treatment trials of complicated skin and soft tissue infections, many of which were foot infections. Additionally, some of these antimicrobials have been proven effective in prospective studies of diabetic foot infections, many of which have been limb-threatening: amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanate, cefoxitin, ceftizoxime, ciprofloxacin, ofloxacin, moxifloxacin, imipenem/cilastatin, ertapenem, linezolid, daptomycin, telavancin, and ceftaroline [6, 48, 59, 62, 66, 70–77]. In comparative prospective (sometimes blinded) trials of treatment for limb-threatening foot infections, the clinical and microbiologic response rates for the studied agents have been similar and no single agent has been proven superior to all others [24, 28, 32]. A recent review examining patients across controlled trials suggested that carbapenem therapy was associated with fewer failures compared with multiple other antimicrobials but also noted the association of MRSA infection with failed therapy [78]. Lauf et al. demonstrated that tigecycline did not meet criteria for noninferiority compared to ertapenem ± vancomycin in the treatment of diabetic foot infection. In this study the cure rates for tigecycline-treated patients with methicillin-susceptible *S. aureus* or *Klebsiella pneumoniae* patients were lower than in those treated with ertapenem ± vancomycin and cure rates in patients with osteomyelitis were low [79].

Empiric antimicrobial treatment should be reassessed between day 3 and 5 of treatment in the light of culture results and clinical response. When patients have responded clinically and therapy is unnecessarily broad spectrum (effective therapy for the bacteria isolated could be achieved by less broad spectrum antimicrobials with possible cost savings, avoidance of toxicity, or a reduction in selective pressure for emergence of antimicrobial resistance), treatment regimens should be simplified based on culture data [24, 28]. If a bacteria resistant to the current therapy has been recovered and yet the clinical response is satisfactory, treatment need not be expanded. This is true particularly for less virulent organisms and gram-negative bacteria; however, it seems imprudent to ignore MRSA. Alternatively, if in the face of an isolate resistant to treatment the response to therapy is unsatisfactory, the wound should be examined for undrained deep space abscess or necrotic tissue that has not been debrided, the adequacy of arterial circulation must be assessed, and because the resistant organism might be a pathogen (rather than colonizing flora), antimicrobial therapy should be expanded to treat this isolate.

The duration of antimicrobial therapy for severe soft tissue foot infection is based upon the temporal response to wound care and antimicrobial therapy. Two weeks of therapy is often effective; however, some recalcitrant infections will require longer courses of treatment [6, 7, 24, 69]. After acute infection has been controlled, antimicrobial therapy that was begun parenterally should be changed to oral therapy with comparable orally bioavailable antibiotics. Even if the ulcer has not fully healed, antibiotics can in general be discontinued when evidence of infection has resolved [24, 28]. Persistent ulcers must be managed with wound care and avoidance of weight bearing so that healing can be achieved and the ulcer eliminated as a portal for later infection. The occurrence of bacteremia, especially if remote sites are seeded, may require extended therapy. Of note, *S. aureus* bacteremia entails a distinct risk for secondary endocarditis as well as for seeding other sites such as bones, joints, and the epidural space [80].

Treatment of Osteomyelitis

The therapy of osteomyelitis, which is one of the most debated and controversial areas in the treatment of foot infection, should coordinate antibiotic treatment with considerations of the surgical debridement of involved bone. Some reports have suggested that osteomyelitis of bones in the foot can be cured or at least arrested for extended periods with minimal debridement plus prolonged courses of antimicrobial therapy [8, 18, 24, 28, 72, 81–84]. Others have suggested that cure rates for osteomyelitis (particularly where bone destruction is evident or bone is visible or detectable by probing the infected ulcer) will be enhanced by aggressive debridement, and even excision of all infected bone when feasible in the fore foot [7, 49, 52, 85].

A careful review of the literature on the treatment of osteomyelitis in the feet of diabetic patients concluded that no particular management strategy could be shown superior. This conclusion emerges because of heterogeneity in treated infections, diversity in the surgical approaches, biases in the selection of treatment modality, variability in antibiotic treatments, and different definitions of outcome [86]. Decisions on when to use primarily medical versus aggressive debridement/resection surgical therapy in treating osteomyelitis is divided and is commonly based on physician experience. Nonsurgical management might be preferred when aggressive resection would lead to unacceptable foot dysfunction, limb ischemia precludes surgery, surgery carries excessive risk or is rejected by the patient, and osteomyelitis is limited to the fore foot (phalanges) with minimal soft tissue infection. If medical therapy fails, surgery may be required. More aggressive surgery is required if infection is life-threatening or may be preferred if there is extensive bone necrosis, foot remodeling is required to correct bony prominences and improve function, the patient wishes to avoid very prolonged antibiotic therapy, or the potential toxicity of required antibiotic therapy can be minimized by aggressive surgery.

Several recent studies have shown promise for the conservative management of diabetic foot osteomyelitis in some settings [87–90]. Lázaro-Martinez et al. recently published a prospective randomized control trial of 52 patients with diabetic foot osteomyelitis comparing antibiotic treatment for 90 days versus conservative surgery with antibiotic treatment for 10 days. Of note, patients with severe infection according to IDSA classification, peripheral arterial disease, charcot arthropathy, glycated hemoglobin >10%, renal insufficiency, or bone exposed at the base of the ulcer were excluded from the study. Cure rates in both groups were similar with no difference in the need for later minor amputation. All patients were treated with oral therapy including amoxicillin-clavulanic acid, ciprofloxacin, and trimethoprim-sulfamethoxazole [88]. Selection of antibiotic therapy is ideally based on the precise microbiology of bone infection. Cultures from curettage of soft tissue deep in the infected ulcer overlying bone may suffice to design therapy when surgical resection of all infected bone is planned, i.e., therapy will be directed at residual soft tissue infection. When bone debridement will not be done or is limited, as in mid-foot or calcaneal osteomyelitis, bone culture to define the microbiology is of paramount importance. Culture of soft tissue adjacent to bone does not adequately define bone microbiology [20]. Additionally, favorable outcome of therapy is more likely using antibiotics based on bone culture [83]. Adequate antibiotic therapy can be achieved by intravenous administration or the use of highly bioavailable oral agents. Specific antibiotic choices are contingent on pathogen susceptibility. Often sequential intravenous to oral therapy is used. The role of local therapy using antibiotic impregnated materials is not established [91].

The duration of antibiotic treatment for osteomyelitis is based upon the amount of residual infected bone and soft tissue (Table 17.5). If all infected bone is resected en bloc, e.g., amputation of a phalange or phalanges and the related distal

Table 17.5 Duration of antibiotic therapy for osteomyelitis of pedal bone

Site/Setting	Duration
Amputation with no residual infection	2–5 days after surgery
En bloc resection all infected bone with residual soft tissue infection	2–3 weeks
Residual infected bone (piecemeal debridement) *Initial intravenous therapy, then consider oral therapy*	≥6 weeks after debridement
Medical therapy or after surgery with residual devitalized bone *Initial intravenous therapy, then consider oral therapy*	3–6 months

Adapted from Lipsky, B.A., et al., 2004 [24]

metatarsals, the residual infection has in essence been converted to a soft tissue process and can be treated accordingly, i.e., for 2–3 weeks [6, 7, 24, 49, 92]. However, in this setting it is prudent to document by proximal bone margin histopathology that all infected bone has been resected. Failure of antibiotic therapy directed at soft tissue has been noted when bone resection was incomplete and residual osteomyelitis was present at the proximal margin.

If debridement removing all infected bone cannot be assured, the management strategy must be altered. Thus when osteomyelitis involves bones that cannot be resected en bloc without disruption of the functional integrity of the foot, and debridement, if done at all, must be done in a piecemeal fashion, pathogen-specific antimicrobial therapy should be administered for a prolonged period (at least 6 weeks) and adequate blood supply to infected tissues must be assured [7, 18, 24, 28, 92]. Very prolonged antibiotic therapy has been used when medical cure is attempted in the setting of residual necrotic infected bone. Therapy has been given for 3–6 months and occasionally for a year [18, 83, 84, 86]. A recent study by Tone et al. questions the need for very prolonged courses of therapy for all patients treated with medical therapy alone. In this study, 40 patients with diabetic foot osteomyelitis received either a full oral course of therapy or a short course of intravenous therapy followed by a transition to an oral agent with good bioavailability. Patients with severe peripheral arterial disease were excluded from the study. No significant difference in outcome was seen in patients receiving 6 weeks compared to 12 weeks of therapy [90]. Although this study and that by Lazaro-Martinez [88] suggest that contiguous osteomyelitis complicating an infected diabetic foot ulcer can be effectively treated with antibiotics alone, it is important to note that both studies were small and that the enrolled patients were highly selected. Thus physicians should be cautious in generalizing from these data. In every setting the need for debridement, the choice of a specific antimicrobial regimen, and the duration of therapy must be individualized and reflect not only local foot findings but also possible concomitant metastatic infection and potential for antibiotic-related adverse events.

When in spite of apparently appropriate treatment infection fails to respond and ulcers to heal, the foot should be reassessed for adequacy of arterial supply, persistence of necrotic soft tissue or bone requiring debridement, presence of an unresponsive or antibiotic-resistant pathogen, or ineffective antibiotic delivery. Patient noncompliance with treatment or non-weight bearing must be considered as well. Therapy should be redesigned addressing defects found in the prior regimen.

Adjunctive Therapy

The effective treatment of foot infection requires far more than the administration of antibiotics that are active in vitro against the implicated pathogens. Optimal therapy involves the integration of appropriate dressings and wound care, control of glucose metabolism, effective debridement, and possibly reconstructive foot surgery. Wound care should include non-adherent dressing products that maintain a moist wound bed, control exudate, and avoid maceration of surrounding skin [13]. Non-weight bearing (off-loading) on neuropathic ulcers whether infected or noninfected is essential for healing. When ischemia is a limiting factor, vascular reconstruction may result in healing and foot salvage [51].

Many possible elements of adjunctive therapy are insufficiently evaluated to warrant inclusion in standard therapy. If a wound fails to demonstrate improvement after 4 weeks of treatment, adjunctive therapies may be considered [13]. Hyperbaric oxygen therapy may facilitate healing but does not impact infection. The role of platelet-derived growth factor and bioengineered skin equivalent in healing has not been fully established. Treatment with granulocyte colony stimulating factor raises the peripheral white cell count and may accelerate slightly the control of a wound infection but has not become a standard component of care. Negative pressure dressings (vacuum-assisted closure or VAC dressings) in controlled trials have been shown to be safe and, in treating surgical wounds, to accelerate granulation tissue formation, reduce the time to wound closure, and yield a higher overall rate of wound healing [93]. Although widely used, they have not been generally recommended and their role in infected diabetic foot wounds is unclear and not currently recommended for routine use [1].

The importance of ulcer healing and the hazards of protracted nonhealing diabetic foot ulcers have been detailed in a recent study [94]. Among 819 patients discharged after hospitalization for initial treatment of a diabetic foot ulcer, there were 172 episodes of life-threatening non-foot infection caused by organisms that were present in the ulcer. These infections, which included bacteremia, endocarditis, osteomyelitis, septic arthritis, deep tissue abscesses, and others, occurred over a 24-month follow-up period. Patients with ulcers that remained open for >145 days and were initially culture positive for methicillin-resistant *S. aureus* (MRSA) were at highest risk. These infections were independently associated with increased mortality with death often occurring within a month of infection. It appeared that efforts to accelerate ulcer healing were protective against these infections. These data suggest that nonhealing ulcers serve as a portal of entry for bacterial invasion, particularly by MRSA, and hematogenous seeding of remote sites. While this was

a retrospective study, the findings suggest that aggressive adjunctive therapy to achieve rapid ulcer healing—including debridement, ulcer excision and primary closure, limited amputations, and early revascularization—could prevent these life-threatening delayed infections and merit consideration in ulcer management plans.

Topical antibiotics and antiseptics have not been demonstrated more effective than standard wound care and may cause local adverse reactions or promote emergence of resistance in bacteria. Accordingly, these have not been recommended [1].

Outcome

The knowledge and skills to achieve an optimal outcome in the treatment of diabetic foot infections often require the collaboration of multiple care providers, including diabetologists, infectious disease specialists, podiatrists, and vascular surgeons. With appropriate care a satisfactory clinical response can be anticipated in 90% of patients with mild non-limb-threatening infection and at least 60–80% of those with moderate or severe limb-threatening infection. Limb-threatening infections may require foot-sparing amputations but salvage of a weight-bearing foot is usually achievable. Vascular reconstruction, especially bypass grafts to pedal arteries which restore pulsatile flow to the foot, decrease major amputations and enable foot-sparing/foot-salvage surgery. Although the clinical science of treating diabetic foot infections has advanced significantly, challenges remain in defining optimal care. Still many foot infections could be prevented, effective therapy provided, and extremities salvaged if current knowledge was more widely applied.

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Topical Wound Care Treatment and Indications for Their Use

18

John C. Lantis II and Juan A. Paredes

Abstract

In this chapter, we will explore the role of various topical wound care strategies, and the evidence, or lack thereof, in support of their use in diabetic foot ulcer patients. The chapter is divided into two major sections that cover Basic and Advanced wound care strategies. The myriad wound care compounds, dressings, and devices are discussed. The chapter concludes with general recommendation for ulcer management based on stage.

Introduction

While it is generally agreed that diabetic foot ulcers (DFUs) should initially be treated with offloading and maintenance of a moist wound environment, if after 4 weeks the wound has not closed by 50%, adjunctive therapies should be pursued. This includes maximizing revascularization and the surgical correction of bony prominences that lead to pressure points. It is noteworthy, however, that while such basic strategies are often discussed, they are not routinely followed in practice. Even the broadly accepted concepts of wide surgical debridement and placement of cellular or tissue-based products (CTPs) for dermal regeneration have not been widely adopted as standard of care. The TIME principal (Tissue, Infection, Moisture balancing, Edge enhancement) has been studied in diabetic foot wounds and lends itself well to the care of such patients [1]. The portions of TIME that address tissue debridement followed by infection control, moisture balance, then edge enhancement have areas that cross over with topical wound care agents in several ways. Topical debriding agents, both mechanical and enzymatic, work in part by direct wound contact. Topical antimicrobial agents such as silvers and cadexomer starch with iodine fall in this category as well. While epithelialization has been shown to be enhanced by a moist wound environment, there are no data to universally support a single topical product for closure of diabetic foot ulcers. Most notably, the well-respected Cochrane review recently (2015) concluded that "there was no clear evidence that any of the 'advanced' wound dressing types was better than basic wound contact dressings for healing foot ulcers. Findings were limited, however, by the small amount of information available (a limited number of trials involving small numbers of participants)" [2]. The conclusion was based on 13 systematic reviews that contained 17 relevant randomized-controlled trials published up to 2013. There were 10 different types of wound dressings evaluated in a total of 37 different comparisons. However, the outcome measures differed significantly between studies and several were deemed to provide low quality evidence. In our opinion, the gravest shortcoming of such studies was a failure to acknowledge that no one dressing type is appropriate for all phases of wound closure. We believe that an ideal approach will ultimately be stepwise and requires combination therapies to achieve wound healing. Unfortunately, the proprietary "corporately owned" nature of current products has not allowed for this type of progressive combination evaluation. For these reasons, the outcome measures used by investigators in the Cochrane's review may have been appropriate, but do not reflect how clinicians currently use such products.

It would seem that clinical efficacy, outcome analysis, and total cost efficiency per area reduction should dictate dressing choices for diabetic foot ulcers. However, it is the absolute unit cost, not cost of care, and clinician preference that undoubtedly play a large influence. Until such questions

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regarding individual dressing types are addressed in a rational and systematic fashion, it will be impossible to present balanced evidence on the best dressings to use. For these reasons, we believe that it is incumbent upon principal investigators and corporate sponsors to develop trials that acknowledge the metachronous nature of wound care, and define a stepwise evaluation of these processes. It should wound care research needs to mimic cancer therapy research.

Role of Dressings

It has been stated that an ideal wound dressing promotes a moist wound environment, provides for mechanical protection, and does not adhere to the wound or CTP (cellular or tissue-based therapy). It should also minimize pain and trauma, provide for absorption of excess exudate, and allow for gaseous exchange. An ideal dressing should also be noncytotoxic to healthy tissue. Finally it should be acceptable to the patient, easy to use, and cost effective [3]. At present such qualities are nigh on impossible to achieve in one dressing. The role of dressing is to keep the wound environment balanced so that it can heal, similar to an acute wound rather than a chronic wound.

In general, we know from early work that epithelialization occurs in a moist, clean, and warm environment [4, 5]. The clinician relies on dressing to create and maintain this environment. There are basically four principals that one applies when choosing the optimal topical dressing for DFUs [6]. If a wound is dry it needs to be hydrated. If the wound produces excessive exudates it requires absorption. If it has necrotic tissue or fibrinous debris, it will need debridement. And if the wound exhibits critical colonization, it needs a topical antimicrobial for an appropriate period of time. There are a host of factors that are important when choosing a dressing including the need to decrease maceration, act as a barrier to bacterial influx, conform to wound shape, produce minimal pain during application and removal, and maintain the wound at an optimal temperature and pH. The clinician is often tasked with balancing the pros and cons of a dressing to maximize what it can deliver. Unfortunately, in many situations, the cost (or lack of insurance coverage) of such an ideal dressing is the limiting factor. One must keep in mind that no one topical therapy is correct through the entire course of a wounds evolution. Therefore, the ideal topical dressing for a diabetic foot ulcer will change during its clinical progression.

Basic Wound Care Strategies

As stated, there is limited evidence to support the use of one topical dressing over another. However, dressings should be evaluated in sequence and as they may play a role in facilitating the TIME concepts of diabetic foot wound care. Therefore, the selection of dressings in a step wise fashion makes more sense for routine clinical practice. Today there are nearly 200 product manufacturers marketing hundreds of brands of traditional (woven and nonwoven) and advanced wound dressings [7]. Combined, there are thousands of wound dressings available.

The Moist Wound Environment and Dressings

A dematologist, Dr. Gilje, noticed that if he covered venous ulcers with strips of adhesive tape spaced 3 mm apart, the portion of the ulcer covered by the tape epithelialized faster. He published this work in 1948. The patients with ulcers covered with moisture retentive dressings 15/23 (65%) healed in 12 weeks [8]. In the early 1960s, George Winter proposed the concept of an optimal local environment for wound healing and the importance of dressings was brought to light [9]. Winter's studies in 1962 compared the rate of epithelialization in a moist versus a dry wound environment. For the moist wound environment he used an occlusive dressing and for the dry environment, wounds were exposed to air. He showed that reepithelialization occurred twice as fast in a moist environment where a crust or scab was unable to form. Himman and Maibach replicated Winter's studies in human subjects and confirmed the results [10]. These findings precipitated an evolution in dressings that were increasingly designed to interact with the wound to provide an ideal environment for repair.

However, high-quality evidence for this strategy has not been presented. As such, best dressing practices are based on guidelines with relatively low levels of support. Even with the tremendous number of new wound care products on the market, gauze continues to be the de facto wound dressing of choice. Studies over many years clearly show that a dressing that retains moisture enough to prevent crust formation allows for faster wound healing, decreases risk of infection, requires fewer dressing changes, and helps keep a control environment that promotes wound healing [11]. Contrary to early concerns, the moist, environment created by occlusive dressings does not lead to increased infection rates. In fact, a retrospective analysis of the literature found a decrease in the incidence of wound infection (on both acute and chronic wounds) with the use of occlusive dressings [12]. In a diabetic foot ulcer, the amount and type of drainage, size, depth, type of ulcer, and condition of the surrounding skin may help guide the wound care provider in selection of the proper dressing. Furthermore, as the wound heals or stalls, reassessment with subsequent change in wound care dressing may be needed. Finally, due to the chronicity of these types of ulcers, cost may also need to be considered.

There are numerous commercially available wound care products on the market. These products offer many benefits

including the maintenance of a moist wound environment, the provision of antimicrobial activity, absorbance of excessive exudate, diminishing inflammatory cytokines that may be toxic to the healing process, promoting growth factors integral to wound healing, and debridement of necrotic and fibrotic tissue. It is important to note that while wound care dressings may provide all of these benefits, they will not offload pressure from the wound site nor can they replace antibiotic therapy in the face of wound infection.

For purposes of reimbursement, dressings have been positioned in several product categories, generally based on their structure or composition. Dressings commonly used in basic wound care strategies include hydrogels, hydrofibers, amorphous hydrogels, moist gauze, nonwoven sleeve dressings, transparent films, hydrocolloids, alginates, hydropolymers, hydroconductives, medicated dressings, impregnated gauze, honey, foams, collagen or extra cellular matrix type, superabsorbents, and combination products. The following section describes products in the category and our experiences with their use in diabetic foot ulcers.

Hydrogels are complex, hydrophilic, organic, cross-linked polymers, consisting of an 80–90% water base, primarily available in a free-flowing amorphous gel. They absorb a minimum amount of fluid and in general are used to keep wounds moist. They can absorb fluid by swelling, but they also can donate moisture to a dry wound, thereby facilitating autolytic debridement and maintenance of a moist wound environment that is thermally insulated. They have also been shown to promote granulation and epithelialization and reduce the temperature of a wound bed by up to 5 °C [13]. Hydrogels are permeable to gas and water but have proven to be a less effective bacterial barrier than occlusive dressings. The main application of these dressings is in hydrating dry wound beds and softening and loosening slough and necrotic wound debris. They are unable to absorb heavy drainage due to their high water concentration and they absorb very slowly. Therefore, they are not useful on bleeding wounds and they generally require a secondary dressing. One benefit is that they can be used on a variety of wound types including pressure ulcers, partial- and full-thickness wounds, and vascular ulcers. Maceration can be of concern, as peri-wound skin areas need to be protected from excess hydration. Among its benefits, hydrogel can be used in conjunction with topical medications or antibacterial agents. The fixed form of hydrogels should not be used in infected wounds. Hydrogels need to be covered with secondary dressings and may remain in place for up to 3 days.

Studies related to hydrogels date back to 1990s. One clinical trial comparing hydrogel to a hydrocolloid looked at pressure ulcers and demonstrated a benefit for the former [14]. Another historic study evaluated hydrogel versus povidone-iodine solution in pressure ulcers which not surprisingly showed superiority for the hydrogel. On the other hand, two studies looking at hydrogel versus clostridial collagenase have shown superiority for the latter [15]. However, hydrogels rarely have shown up in diabetic foot trials. They are often used in conjunction with cellular or tissue-based products. Hydrogel sheets are three-dimensional lattices made up of a hydrophilic polymer such as polyvinylpyrrolidone. This property makes them useful for burn treatment or large superficial abrasions. When compared with no coverage for wounds, hydrogels and hydrocolloid dressings have been reported to increase epidermal healing by approximately 40% [16]. Hydrogel dressings are not very useful for diabetic (neuropathic) foot ulcers unless the wound is very shallow and only drains minimally. However, they are useful for excoriation or cracking caused by dry skin in this patient population. Hydrogel dressings are also useful in treating painful inflammatory ulcers and other superficial wounds caused by trauma.

Included in this category, though not true hydrogel sheets are biocellulose wound dressings. A biocellulose wound dressing made from purified bacterial cellulose has been introduced that can both deliver or absorb moisture. This dressing accelerates autolytic debridement while providing a protective seal over the wound similar to a blister roof [17]. This dressing can deliver a subtherapeutic dose of silver as well.

Hydrofiber dressings have been evaluated in DFUs and found to be effective in exudate management and the promotion of healing. Hydrofibers are made of carboxymethylcellulose (CMC); they form a gelatinous material when they come in contact with serosanguinous exudates from the wound bed. Hydrofiber dressings rapidly absorb exudates and have a large absorptive capacity (approximately 2–3 times greater than alginates) [18]. Obviously, they are indicated for heavily draining wounds or when extended wear is required. Hydrofibers can also contain silver with the intent to reduce the wound's bacterial burden; they also reduce the levels of matrix metalloproteinases (MMP) and the amount of debris on the wound. In patients with neuropathic ulcers that are being treated with a total contact cast, hydrofiber dressings can be kept on for 7 days. It has been our experience that hydrofiber dressings containing silver help to reduce wound odor [19].

Amorphous Hydrogels come packaged in tubes, spray bottles, or foil packets, and they may also be impregnated into gauze. In the amorphous hydrogel the hydrophilic polymer has not been cross-linked and therefore remains in a more aqueous, gel-like state. The primary ingredient is water and it can dry rather quickly if not covered with a semiocclusive or occlusive dressing. Several amorphous hydrogels contain additives such as collagen, calcium alginate, or CMC in order to be more absorptive. Like a moisturizing agent, amorphous hydrogels will donate moisture and can be useful to soften dry eschar or callous.

Moist Gauze has traditionally been used as the control arm in diabetic foot ulcer healing trials. Moist-to-moist gauze dressings and effective offloading are considered to be standard of care for diabetic, neuropathic foot ulcers [20]. The dressing regimen consists of daily changes with dry gauze as the secondary dressing and anchored with an adhesive tape or bulky rolled gauze bandage. This dressing regimen is useful for uncomplicated superficial ulcers that can be offloaded easily with a healing sandal and the use of crutches. It should be avoided in large exudative ulcers, if it affects the fit of the treatment shoe, and with the use of a total contact cast.

Nonwoven Dressings such as sleeve dressings or Telfa® non-adherent brand dressings can serve the role of gauze. Since these dressings are not very absorptive, the same rules apply as when using gauze. Nonwoven island dressings with an adhesive border are useful for very superficial minimally draining wounds. Be sure that the adhesive is safe for use with diabetic skin and does not reinjure upon removal.

Foam Dressings combine occlusion and moist wound healing with some degree of absorption. These wound dressings are made from foamed urethane or another polymer creating open compartments (open cell foam) that house the exudates. To a certain degree, absorption by foam dressings depends on the size and number of open cells generated during the foaming process. Most foam dressings are between 0.5 and 1 cm thick. Foams have a thin urethane film covering the outer surface. This polymeric film over the top maintains the moist environment by regulating the moisture vapor transmission rate (MVTR). The film covering also provides a seal to water and exogenous bacteria. Foam dressings may have an adhesive coating over the wound contact layer or may have an island configuration where the foam is at the center and the perimeter provides the adhesive contact layer. Foams dressings may also contain additives such as surfactants, glycerin or superabsorbents aimed at improving the function of the foam. There are also foam dressings that are impregnated with antibacterial agents such as silver or polyhexamethylene biguanide (PHMB). Foam dressings are often used as primary dressings (in direct contact to the wound), however in the setting of using a topical debriding gel, antimicrobial gel or topically applied growth factor foam dressing become a secondary dressing. Foam dressings are appropriate for diabetic ulcers with moderate to heavy drainage, or for ulcers with minimal drainage where the dressing can remain in place for 3–7 days. Unless the foam is an island dressing where adhesive covers the perimeter, a secondary dressing, adhesive tape or a bandage will be necessary to anchor the product. The foam design will imbibe wound fluid and keep it away from the wound. For chronic wounds (or wounds that are >2 months old) this is a desirable attribute as it has been shown that chronic wound fluid may be harmful to cells and provisional matrix. Foam dressings also provide a cushion that may be helpful to protect the wound from friction or trauma.

Multiple companies have proprietary interests in foam dressings, therefore there have been numerous studies in these dressing types, however very few of these are in DFUs. The properties of foams allow for the absorption of fluid as well as the space occupation and the near tissue apposition. We use foams as a secondary dressing over many active topical dressings. However, foams have not been well studied in diabetic foot wounds, and therefore we need to extrapolate from other studies like the FOUNDers trial where foam is used over a cell or tissue based therapy or a hydrogel [21]. The intrinsic properties of some foams provide significant advantages. In particular, they provide benefits in terms of their ability to provide a moist wound environment and promote wound healing, provide mechanical protection, non-adherence to the wound or CTP, minimize pain and trauma, absorb excess exudate, and allow for gaseous exchange. They also are non cytotoxic to healthy tissue, and possibly contain antimicrobial activity. They are acceptable to the patient, easy to use, and cost effective. Foam dressings have been evaluated primarily in venous leg ulcers, but these have been company sponsored and as such, concerns for bias must be high. However, open cellular foams (ones that fill space when moistened) have been shown to absorb more fluid, resulting in fewer dressing changes and lower costs than closed cellular foams [22]. Based on these near ideal qualities, an open cellular foam is good as a secondary dressing in DFUs as it allows the primary dressing to have very good apposition to the wound bed. We find that closed cellular foams work better than active agents such as topical enzymatic debriders and growth factors, since they do not expand into the space pushing the gel out of the wound

In DFUs, if we are not employing wide sharp debridement followed by dermal regeneration in combination with offloading, we try and use topical dressings for specific indications. After local debridement, we employ a topical antimicrobial dressing, followed by a topical permissive maintenance debridement therapy and then topical pro-epithelialization strategy in the form of topical growth factors. However, next to offloading (total contact casting and weekly debridement) simply keeping the wound moist may be the most important item.

Transparent Film Dressings were first introduced as IV site dressings or surgical incise drapes. They were used as wound dressings in the late 1970s and have been shown to promote the healing of partial thickness minimally draining wounds [23]. We find that transparent film dressings are not useful for the treatment of diabetic foot ulcers mainly because they do not have any absorptive capacity. The exudates tend to remain in contact with the wound and surrounding skin causing maceration. In addition, frequent strike-through eliminates the edge seal and exogenous bacteria can gain

entry. For superficial abrasions, skin tears, and diabetic bullae, transparent films are useful when used together with a topical antibiotic agent.

Hydrocolloid Dressings are the direct descendants of ostomy devices and barrier products. Hydrocolloid dressings are completely airtight and do not allow the transport of oxygen or other gases. In the 1970s wound-healing research with hydrocolloids dispelled the old notion that "the wound should be allowed to breathe" [24]. From these studies it became obvious that the oxygen necessary for wound repair came from the blood and that atmospheric oxygen often harmed or delayed the healing process [25]. On the other hand, its occlusive nature has made it a questionable dressing for DFUs. Mixing a hydrocolloid such as CMC with gelling agents such as gelatin and combining them with an adhesive elastomer such as isobutylene create these dressings. Hydrocolloids are dispersions of discrete particles around which water molecules and solvated ions form a shell-like structure. Fluid absorption occurs principally by particle swelling and enlargement of this structure. The hydrocolloid mass of these dressings consists of gum-like materials such as guar or karaya, sodium CMC, and pectin bound by an adhesive such as polyisobutylene. Certain hydrocolloid formulations can adhere to wet surfaces (wet-tack) because of particle swelling and phase inversion. When placed over a moist wound the immediate wound contact area dissolves in time to form a semisolid gel that allows for dressing removal without reinjury. Exudate absorption by most hydrocolloid dressings results in the formation of a yellow/light brown gelatinous mass that remains covering the wound upon dressing removal. This may be irrigated from the wound and should not be confused with pus. As hydrocolloids and gelatin decompose over the wound, there may be a characteristic odor that resolves once the wound has been cleansed. Hydrocolloid dressings are particularly useful when autolytic debridement is desirable. The wound environment created under a hydrocolloid dressing is acidic (pH 5) and has been shown to inhibit the growth of pathogens such as *P. aeruginosa* and *S. aureus*. However, their occlusive nature concerns many in the treatment of potentially colonized DFUs, therefore they are fundamentally contraindicated in the setting of a DFU.

Alginate Dressings are the calcium salts of alginic acid (derived from brown seaweed) that have been spun into a fiber. These fibers can be configured into multiple forms that are available as compressed nonwoven sheets or bound into ropes. When wound fluid contacts the calcium alginate, the sodium in the fluid replaces the calcium in the alginate increasing the viscosity of the fluid producing a gel (sodium alginate). Alginates are bioerodible and will gradually dissolve with moisture over time; therefore, the greatest advantage of the alginate dressings is their absorptive capacity. Alginates are ideal for heavily draining wounds. If used appropriately, they can significantly reduce the number of dressing changes required. They are useful in superficial wounds since they are manufactured in sheet form, as well as ribbon or cord for packing deeper wounds and tracts, including those characterized by infection, tunnels, sinus tracts, or with exposed tendons. If used in wounds that drain minimally, the fibers will dry out and will adhere to the wound bed. The secondary dressing is important and one should be chosen that helps to keep the gel moist. Alginates have been reported to have hemostatic and bacteriostatic properties [26]. Alginate dressings are also available with the topical antibacterial silver (controlled-release ionic silver).

Hydropolymers are foamed gels that wick exudates away from the wound to the upper layers of the pad. The backing material has a very high moisture vapor transmission rate (MVTR) and allows for the evaporation of excess fluid. Hydropolymer dressings are available with silver as well. These dressings are useful for moderate and heavily draining wounds or when the dressing needs to remain in place for an extended period of time. There is some evidence that these dressings can decrease matrix metalloproteases at the wound interface.

A Hydroconductive Dressing absorbing system is structured by two types of absorbing layers that work on a cross-action style that simplifies the movement of large quantities of exudates and bioburden away from the wound bed and through the dressing. The dressing can withstand 30–50 times its own weight in fluid, without breaking down and leaving dressing residues on the wound, even after 7 days of wear time. During multiple trials hydroconductive dressings have been shown to significantly reduce the bioburden together with the components required by bacteria to proliferate in the wound. The wound bed shows a substantial reduction in MMPs, bacterial counts, including serious bacteria like the methicillin-resistant Staphylococcus aureus (MRSA). This is accomplished by attracting the bacteria and its components from the wound base into the dressing.

Medicated Dressings are devices that contain an agent (usually an antimicrobial) in order to supplement its function. Recently there has been great interest in the use of silver-containing dressings. The antimicrobial properties of metallic silver have been used empirically for thousands of years and a great deal has been published regarding its mechanism of action, anti-inflammatory properties, toxicity, and historical background. Many dressings have been introduced that contain silver in a variety of different forms. There are dressings that contain a silver coated polyethylene membrane, ones that contain silver-impregnated activated charcoal cloth, alginates, foams and hydrocolloids containing silver, microcrystalline silver on the adhesive portion of a transparent film, silver powders, and even an amorphous hydrogel containing silver. The antimicrobial properties of several of these silver-containing dressings have been studied and shown to be effective against bacteria (including methicillin-resistant

S. aureus (MRSA) and vancomycin-resistant enterococci (VRE)), Pseudomonas aeruginosa, fungi, viruses and yeast [27]. Interestingly the silver content and antimicrobial activity of the various dressings varies considerably and most are limited to the superficial organisms located on the wound bed. A recent systemic review of 26 randomized-controlled trials did not find evidence of increased wound healing on uninfected wounds with silver [28]. More specific to diabetic wounds, a systematic review examining the efficacy of silver in healing diabetic foot ulcers did not find any studies that met the inclusion criteria—a randomized control trial with diabetic ulcers comparing silver dressings to a control—and concluded that more trials are needed to determine effectiveness [29]. Polyhexamethylene biguanide (PHMB) has been used as an antimicrobial agent by the contact lens industry for years. Recently, several manufacturers have incorporated this antimicrobial agent into their wound dressings, since they are active against a broad spectrum of bacteria, fungi, molds, and yeasts. A biocellulose wound dressing containing PHMB has recently been introduced and PHMB has also been impregnated into gauze and nonwoven.

Iodine preparations have been criticized in the past because of their cytotoxicity. However, in cadexomer iodine formulations the iodine is released in quantities that are not harmful to cells. Cadexomer iodine is available in an absorbent gel and also as a paste dressing. Cadexomer iodine has been studied in both venous ulcers [30] and diabetic foot ulcers [31] with favorable results, but these studies had relatively small sample populations. These dressings have to be utilized with caution in certain groups of the population, including: patients with thyroid disease, iodine sensitivity, pregnant women and breast-feeding women, children or neonates. A randomized-controlled clinical trial of cadexomer iodine for the treatment of diabetic foot ulcers has not been done to date. The commercially available cadexomer iodine dressing has on balance of the greatest amount of actual data showing the log kill reduction of bacteria (both planktonic and biofilm protected).

Combination Products/Impregnated Gauze Dressings are gauzes and nonwoven dressings that are incorporated with agents that affect their function. Dressings have long been used as drug delivery devices. The agents most commonly used include saline, oil, zinc salts, or petrolatum, Vaseline®, Aquaphor®, or (bismuthtribromophenate) bacteriostatic agents. Gauze or polyethylene may also be impregnated with salts and inorganic ions that appear to decrease the harmful effects of MMPs in chronic wounds.

Honey, a sugar solution modified by a honeybee from nectar, has been used to promote wound healing since ancient times. Due to its acidic pH, low water content, and hydrogen peroxide secretions, honey is less likely to develop resistance against organisms in a wound [32]. Mostly used medicinally in tube or gel form, honey is applied either to gauze or directly to the wound and changed daily. These dressings have also been shown to decrease the amount of slough/exudates and malodor in the wound bed, while at the same time having and anti-inflammatory and immune-modifying effect. As the wound secretions lessen, the number of required dressing changes decreases. A controlled, comparative study between honey and povidone iodine for Wagner type II diabetic foot ulcerations in 30 patients did not find statistical significance between the two groups in healing time [33]. However, a recent systemic review found insufficient evidence for the use of honey in clinical practice for chronic, diabetic wounds [34]. More research is needed to accurately determine the effectiveness of honey on wound healing. In unpublished work from our program we could not document a reduction in bacteria, interleukin 1, nor tumor necrosis factor in chronic wounds treated with honey, while we did see a significant redcution in wound area.

Secondary Dressings

Gauze has been selected as the secondary dressing in many clinical trials including PDGF trials, however the comparator in those trials has been gauze without the active agent. In our practice we tend to use dressings that have the "ideal properties" of moisture balance; such as foams [35]. While gauze is inexpensive and conforms well, it also adheres to wound, does not prevent bacterial translocation, and it only absorbs its own weight in fluid. Wet to moist (with 0.9% sodium chloride solution) gauze dressing have been written about extensively but it is usually practiced as wet to dry dressings which have been shown to be inferior to gauze dressing with topical clostridial collagenase in one diabetic foot study [36]. In addition others have cited foreign body reactions, repetitive trauma and wound cooling as negatives of gauze. Bacterial translocation and increased infection rates have also been commented on when compared to hydrocolloids and foams [37, 38]. There is really no clinical data, in part due to lack of a profit motive, to support the clinical efficacy of impregnated gauze in the diabetic foot wound. Its only application may be to decrease adherence on Wegner Grade I wounds or over CTPs. The non-absorbent nature of these dressings in the average DFU patient makes these dressings of little utility on the treatment of the DFU. Transparent film dressings are thin flexible transparent sheets with adhesive backing, composed of polyurethane or co-polyester. They are permeable to water vapor, oxygen, and carbon dioxide but impermeable to bacteria and water. While they provide a moist healing environment they are not advisable over potentially critically colonized wounds and there is no clinical support for their use in DFUs.

For our group, foam dressings are made from a polyurethane base and are permeable to both gases and water vapor. Their hydrophilic properties allow high absorptive properties while they also provide thermal insulation. These highly versatile dressings are indicated for wounds with moderate-to-heavy exudates, granulating or slough covered partial- and

full-thickness wounds, donor sites, ostomy sites, minor burns, and diabetic ulcers. They are not recommended in dry or eschar covered wounds and arterial ulcers due to their ability to dry wounds further. They can be left in place for up to 4–7 days, but should be changed once saturated with exudates. Their composition makes them atraumatic upon removal. If changed daily, they can also be used on infected wounds [39].

Advanced Wound Care Strategies

Topical Biologically Active Compounds

Growth Factors

Growth factors (GFs) are polypeptides that have several functions in the human body. Through their involvement in cell proliferation, chemotaxis, extracellular matrix formation, angiogenesis, and wound contraction, they play fundamental roles in wound healing. When an acute tissue injury occurs, several cell types migrate to the site of injury to control/stabilize the wound. One of the most important cell types during this process are platelets. When blood vessels are disrupted during tissue injury a substantial numbers of platelets enter the site of injury to release growth factors and cytokines including platelet-derived growth factor (PDGF) and transforming factor-β1 (TGF-β1). These and other growth factors are chemotactic for a number of cell types critical to the repair process, such as macrophages, fibroblasts, and endothelial cells. Later, during the proliferative phase of wound repair, several growth factors, including vascular endothelial growth factor (VEGF), fibroblast growth factors (FGFs) and PDGF and TGF-ß isoforms provide a potent stimulus for angiogenesis and for fibroblasts to synthesize key extracellular components, while the growth factors are actually also dependent upon proteoglycans and glycosaminoglycans for their binding to enhance function.

Growth factor therapy is a promising approach to wound healing that may work by addressing the deficiency in the chronic wound. However, data to support the clinical utility of growth factor therapy has been lacking. The transient nature of growth factors activity may contribute to this phenomenon as it is possible that we have not yet found the correct delivery system. Another reason is that the microenvironment of these chronic wounds is very hostile to proteins, and that breakdown of peptides by proteases is very likely. Therefore it is possible that the success of PDGF in diabetic ulcers may be due to the persistence of biologic activity of the peptide in the wound microenvironment [40]. A third reason is that resident cells in chronic wounds have undergone a phenotypic change and as such do not respond to GFs. There is evidence that fibroblasts from chronic wounds, including diabetic ulcers, are not able to respond to certain growth factors [41]. Therefore, repeated debridement of tissue from around the wound, as has been advocated for the use of PDGF in diabetic ulcers [42] (Fig. 18.1 [43]) this intervention may remove these unresponsive cells and allow peptides to function as they should.

The Cochrane review recently identified 28 growth factor trials looking at 2365 patients with neurologic, vascular, or combined DFUs conducted in 10 countries. The trials assessed 11 growth factors in 30 different comparisons. Growth factor compounds reviewed included platelet-derived wound healing formula, autologous growth factor, allogeneic platelet-derived growth factor, transforming growth factor β2, arginine-glycine-aspartic acid peptide matrix, recombinant human platelet-derived growth factor (becaplermin), recombinant human epidermal growth factor, recombinant human basic fibroblast growth factor, recombinant human vascular endothelial growth factor, recombinant human lactoferrin, and recombinant human acidic fibroblast growth factor. This review demonstrated that any growth factor (657 patients) compared with placebo or no growth factor (482) increased the number of participants with complete wound healing, (345) 53% versus (167) 35%. However, this was predicated on 12 trials, and these numbers were primarily based on two trials of platelet-derived wound healing formula 36/56 (64.28%) versus 7/27 (25.92%); and recombinant human platelet-derived growth factor (becaplermin) 205/428 (48%) versus 109/335 (33%). Interestingly, there was no clear evidence of a difference between any growth factor and placebo or no

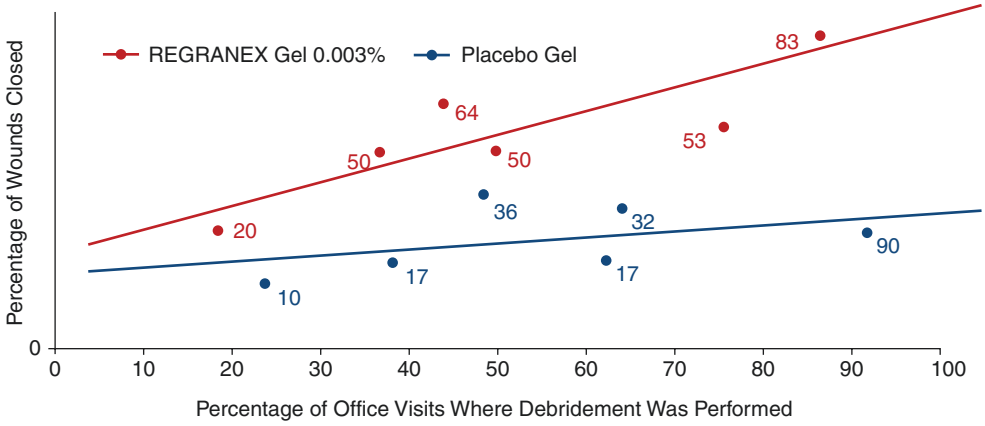


Fig. 18.1 Sharp debridement is important to successful growth factor therapy

growth factor when looking at the effect on minor amputation; major amputation was not well assessed across the trials [44]. The reported studies likely probably represent only a partial list as many unsuccessful trials are not published.

In gene, therapy nucleic acids are used for therapy. There are three different concepts in gene therapy for chronic wounds: (1) synthesis of human recombinant growth factors by gene therapy techniques, (2) ex vivo transfection of cell cultures (fibroblasts, keratinocytes) with growth factor DNA and subsequent transplantation of transfected cells on chronic wounds, and (3) in vivo transfection with growth factor DNA, e.g., gene gun, liposomes, and viral vector. Clinical studies on gene therapy for diabetic foot ulcers are only available for the local application of human recombinant PDGF-BB growth factor. Meta-analysis shows there is a low but significant effect of PDGF-BB on neuropathic diabetic ulcers, leading to an increase of healing by 10–15% within 20 weeks of treatment. To date most gene therapy trials have been targeted at ischemic foot ulcers that happen to be diabetic, usually with freedom from amputation as an endpoint, and not wound closure. The VEGF gene therapy trials to date fall into this category and the authors can find two Phase III trials that are active at this time, however inclusion criteria require ischemia to be present.

The results from a Phase 1/2 study of a replication-defective adenovirus encoding human platelet-derived growth factor (PDGF)-B formulated in a bovine collagen (Ad-5PDGF-B; 2.6% collagen; GAM501) gel for non-healing neuropathic diabetic foot ulcers was reported in 2009. The primary objectives of the study were to evaluate the safety, maximum-tolerated dose, and preliminary biological activity of GAM501. Fifteen patients received one of three different dose levels of GAM501, or up to four administrations of GAM501 at 1-week intervals. All patients received standard of care treatment including debridement and were required to wear an offloading shoe. GAM501 was found to be safe and well tolerated with no evidence of systemic or local toxicity at all doses so no maximum-tolerated dose was reached. Ten of the twelve patients that completed the study were closed at 3 months [45]. Interestingly, no Phase 3, clinical trial evidence can be found on the government websites; therefore this obviously did not move forward as a practical therapy. To date these therapies do not have a clear clinical path or efficacy data that supports their use in the closure of the DFU.

Currently, the only GF approved by the FDA for diabetic ulcerations is Becaplermin (rhPDGF-BB). Initial evaluation of rhPDGF-BB effectiveness on chronic wounds was performed in decubitus ulcers [46, 47]. In both studies, ulcers treated with the higher dose of rhPDGF-BB demonstrated increased wound closure rates and greater reduction of wound volume. However, complete wound closure was not a primary endpoint in either study, raising questions as to the ability of rhPDGF-BB to effect wound closure.

As a result of the early promising data from decubitus ulcers, four prospective, randomized, double-blinded studies of rhPDGF-BB were performed on diabetic neuropathic foot ulcers (Fig. 18.2). Patients were treated with rhPDGF-BB at a dose of 2.2 μg/cm^2, CMC, or vehicle alone for 20 weeks or until complete wound closure occurred. Results from this study demonstrated that 48% healed following treatment with rhPDGF-BB (Case 1 [48]) while only 25% healed with vehicle alone ($p < 0.01$). The median reduction in wound area was 98.8% for rhPDGF-BB treated patients but only 82.1% for those treated with vehicle.

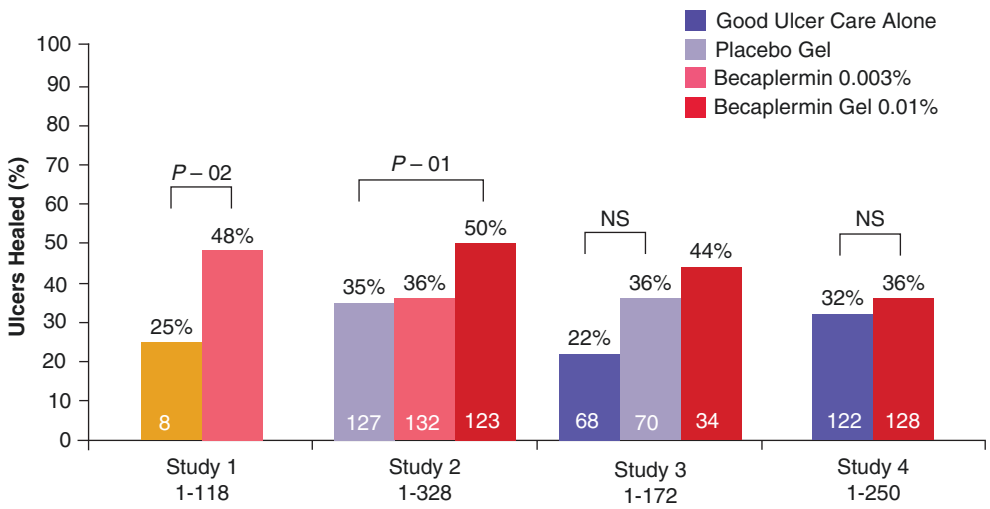
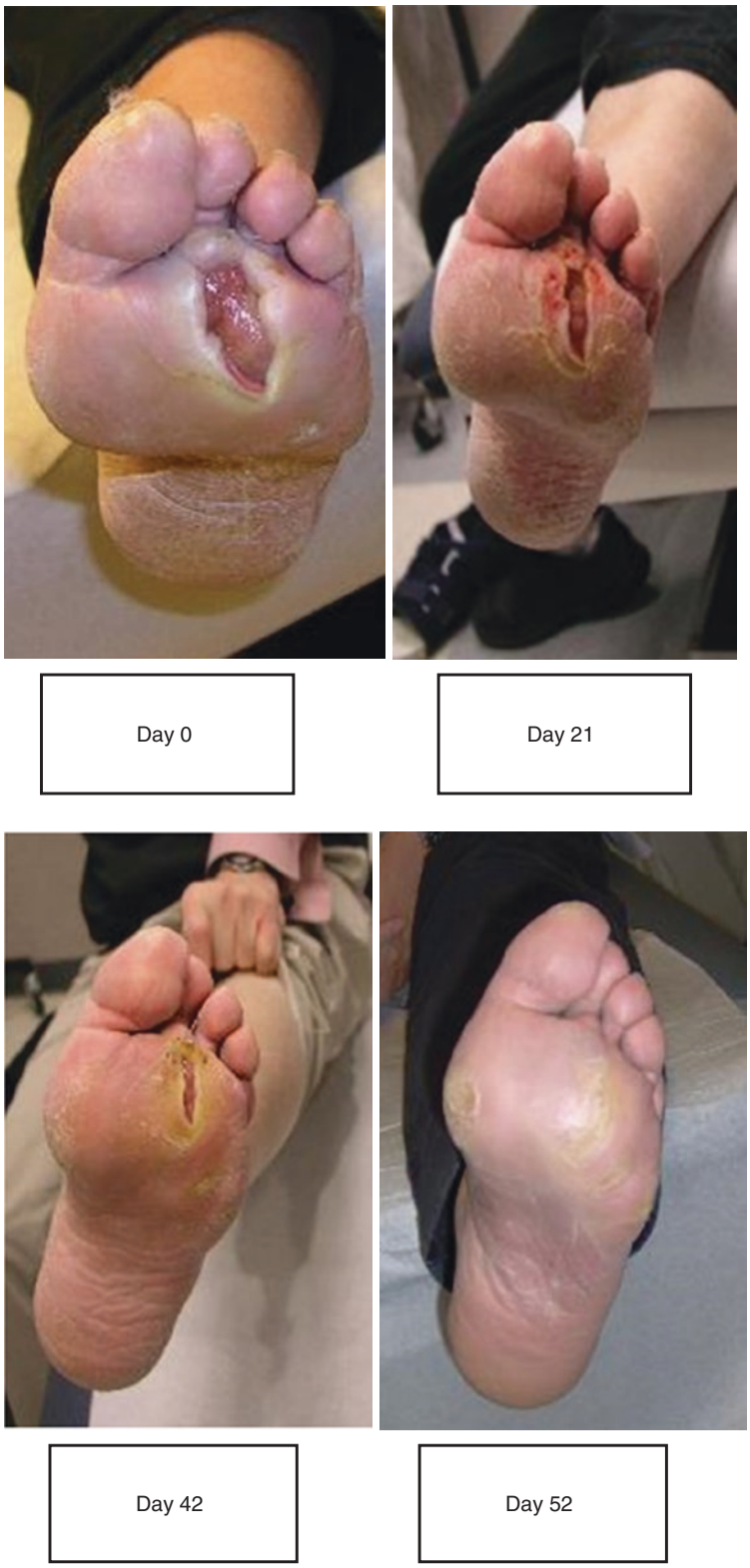


Fig. 18.2 rhPDGF-BB path to FDA approval

Case 1 Response to recombinant Platelet Derived Growth Factor



There were no significant differences in the incidence or severity of adverse events in either group. This was the first clinical trial to suggest that a growth factor, rhPDGF-BB, could be applied topically and be effective and safe in accelerating the healing of chronic wounds in humans (Fig. 18.3) [49]). Despite its promise, it should be noted that judicious use of Becaplermin should be performed in concomitant malignancy, as a recent black box warning

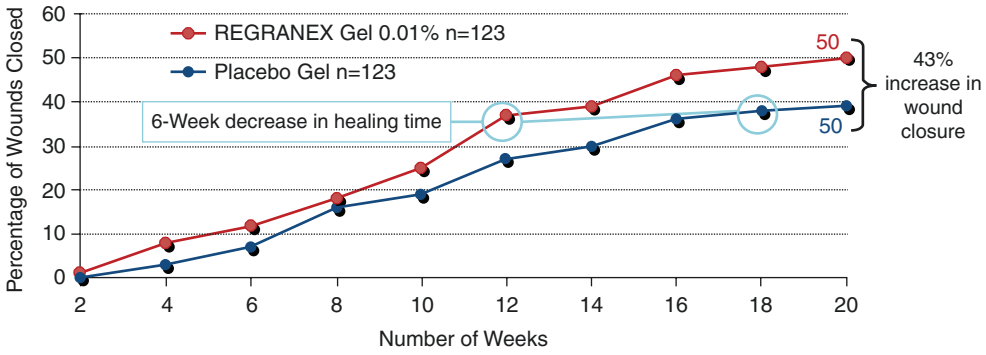


Fig. 18.3 Seminal rhPDGF trial results

was issued by the FDA as a result of evidence of increased mortality from malignancy when using three or more tubes [50]. We generally believe that for small diabetic foot wounds that are undergoing weekly debridement we can expect wound closure or 50% at 5 months when combined with good offloading. While our own data has shown that the average diabetic foot wound that heals with rhPDGF-BB requires only 1.7 tubes [48].

Collagenase

Debridement techniques used in clinical practice for wound bed preparation vary from passive moist dressings ("autolysis") to active surgical, enzymatic, and mechanical debridement. Clostridial collagenase ointment (CCO, Collagenase Santyl® Ointment, Smith & Nephew, Hull, UK) is the only enzymatic agent approved by the US Food and Drug Administration (FDA) for debridement of wounds and burns. CCO has been shown to specifically and preferentially digest native collagens without harming healthy tissue and effectively removes nonviable debris. Although debridement of wounds is thought to be a key element in wound bed preparation that leads to improved healing, there is a paucity of clinical data from well controlled trials to confirm this widespread belief, or shed light on what forms of debridement are most effective.

The largest single study on the effect of CCO with serial (weekly) sharp debridement on DFUs looked at 55 subjects with diabetes mellitus type 1 or 2 and a neuropathic, nonischemic foot ulcer who were enrolled into a randomized, controlled, multicenter trial [51]. Serial sharp debridement without adjunctive CCO was used in the control group. Various standard care therapies thought to support debridement by endogenous proteases were selected at the discretion of the investigators for use in the control group. The primary outcome measure of this trial was the percent change in ulcer area from baseline to the end of the debridement/treatment period (EOT) and at the end of the study after 6 weeks of follow-up (EOS). Wound area decreased relative to baseline for both the CCO group (−68%, −61%) and the control group (−36%, −46%) at EOT and EOS, respectively. While the inter-group differences did not reach statistical significance, wound area was significantly decreased from baseline at both EOT and EOS for the CCO ($P < 0.001$) but not for the control group. On average, ulcers treated with serial sharp debridement plus adjunctive CCO decreased in size more rapidly than ulcers treated without adjunctive CCO debridement.

In addition to this publication there is a larger publication in press, which includes the Motley trial, a pooled data analysis of four randomized trials that compared clinical effectiveness of CCO to standard care (SC). It includes a total of 174 adult patients with DFUs who underwent treatment with CCO or SC for 4 or 6 weeks. Assessments included wound area reduction, wound bed status, and time-to-closure. Mean wound area reduction was numerically greater for CCO than SC at end of treatment (EOT) (−43 vs.-19%). At end of study (EOS) following 6 or 8 weeks posttreatment, these values were −55% and −25%, respectively. Similar EOS results were seen in the subgroup of plantar surface ulcers (−56 vs. −10%, $P = 0.05$) and wounds assessed as "low necrosis" (≤25% necrotic) at baseline (−64 vs. −20%). When rapidly healing ulcers were excluded from the analysis, the difference in ulcer area reduction was even greater for CCO compared to SC at EOS (−53 vs. −7%, $P = 0.05$). Active CCO therapy was associated with greater reduction in wound size than any of the passive or mechanical SC modalities. This was particularly evident when used in conjunction with sharp debridement and for slow healing ulcers, larger size, or plantar surface wounds [52]. Therefore, collagenase treatment may have a role in wound bed preparation.

Bromelain

Bromelain is a mixture of various endopeptidases and other enzymes, such as phosphatase, glucosidase, peroxidase, cellulase, and escharase, which are derived from the fruit or stem of pineapple [53]. Fruit and stem bromelains are prepared differently and their compositions differ. Bromelain is applied as a cream (35% bromelain in a lipid base) and typically for relatively short periods (4 h) [54, 55]. While a small clinical study has shown that bromelain is a debriding agent that does not damage surrounding healthy tissue and has no significant adverse effects, the mechanism of action is still

unknown. At present bromelain is only available outside the United States [56]. To date it has not been evaluated in DFUs and its first trial in the United States will probably be in venous leg ulcers.

Matrix Metalloproteinase (MMP) Reducing Compounds

Matrix metalloproteinase balance is likely necessary in the chronic wound bed. Without them, important enzymes will not be activated and keratinocytes will not migrate. An overabundance of MMPs, however, will lead to the breakdown of the ECM and inactivation of growth factors. New approaches include the topical treatment of chronic wounds with agents that reduce the synthesis of matrix metalloproteinases (MMPs). These include products with a mixture of metal cations, and treatment with unique proteins (amelogenin) that can replace the corrupted ECM [57, 58]. There are several different MMP modulating dressings, however not many have been studied in patients. Promogran (Systagenix) is a collagen-based DFU treatment that chemically modifies the wound environment by acting as a protease inhibitor. Promogran is a dressing that is 55% collagen and 45% oxidized regenerated cellulose, both of which work to bind and inactivate MMPs and elastase within the wound bed to assist with the release of positive growth factors. Promogran/Prisma (Systagenix) is a version of Promogran that helps reduce bacterial growth with the addition of 1% silver [59, 60].

This dressing has been actively studied in DFUs. A total of 276 patients from 11 centers were enrolled in the study. Patients were randomized to receive Promogran (n = 138) or moistened gauze (control group; n = 138) and a secondary dressing. Dressings were changed when clinically required. The maximum follow-up for each patient was 12 weeks. After 12 weeks of treatment, 51 (37.0%) Promogran-treated patients had complete wound closure compared with 39 (28.3%) in the control group, but this difference was not statistically significant (P = 0.12). Of note patients and investigators expressed a strong preference for Promogran compared with moistened gauze. Of note studies of MMP testing devices show that if you treat a wound that test high in MMPs with a MMP deactivating dressing they heal significantly better than those wounds that are high in MMPs but do not receive such therapy. On the other hand the same data set shows that only approximately 50% of wounds test high for MMPs [61].

One study that included the products Puracol and Promogan/Prisma included DFUs (but very few). The authors compared the efficacy of 2 MMP balancing dressings: a sodium carboxymethylcellulose/1.2% ionic silver (CMC), which theoretically reduces bacteria by providing silver ions, versus a bovine native collagen (BDC)/ionic silver dressing, which also delivers silver ions in an aqueous environment. Both CMC and BDC silver dressings appeared to have statistically similar efficacy regarding the rate of wound healing and little impact on the actual bioburden in chronic lower-extremity wounds. Although the BDC dressing showed a higher absolute rate of wound closure, neither technology demonstrated a statistically significant difference in wound closure rate when corrected for initial wound size [62].

Such dressings probably should be used on wounds that have been tested and found to have high MMP levels. In addition, there are anecdotal reports that they may be beneficial as bolsters/secondary dressings for CTP therapies in situations where MMPs can be disruptive to growth factors and cell ingrowth.

Cellular and Tissue Based Therapies

We were initially tasked with writing about Living Skin Equivalents however we believe that this has become an antiquated concept. Our center currently uses the term Cellular and Tissue Based Therapies [CTPs] to define such products and we have further subdivided them into Living Human, Engineered, Xenograft ECMs, Allograft ECMs, and Amniotic subcategories.

Living Human Cellular Derived Products

There are three products other than rPDGF that actually have undergone the rigorous USFDA-Pre-Market Approval (PMA) process for the treatment of DFU; these are Apligraf®, Dermagraf®, and Omnigraft®. None of the other products on the market have had to vault such a high bar. The PMA process is like an approved New Drug Application (NDA)—is, in effect, a private license granted to the applicant for marketing a particular medical device.

Apligraf®

Apligraf® (Organogenesis, Canton, MA) is a composite, bilayer skin substitute derived from human neonatal foreskin fibroblasts and a bovine collagen scaffold. The main application of Apligraf® has been DFUs and VLUs. The FDA has cleared this product for use during standard care for the treatment of full-thickness neuropathic diabetic foot ulcers of greater than 3 weeks that are not responding to conventional therapy and which extend through the dermis but without tendon, muscle, and capsule or bone exposure. In a prospective, randomized, controlled trial in 2001, it was demonstrated that DFUs treated with Apligraf® healed significantly faster than those treated with a standard dressing regimen [63]. An analysis of skin substitutes utilized in the treatment of DFUs conducted in 2016, showed that Apligraf® had a 58% closure rate of DFUs over 90 days, while at the same time being less costly than other CTPs in its class (e.g., Dermagraft) [64]. Similar numbers to the ones published in 2017 by Zelen, et al. where

the healing rate for Apligraf® was around 47.9 days for the subjects in the study, better than the rate 57.4 days obtained in the standard wound care group [65]. Apligraf® has had numerous other therapies compared to it; most recently it was compared to dehydrated amnion/chorion membrane allograft (dHACM) (EpiFix®-MiMedix; Marietta, GA)—where in DFUs it closed 48% of DFUs at 12 weeks (vs. 28% for Dehydrated Amniotic Membrane (DAM)), and 72% at 24% (vs. 47% for DAM). In addition those that closed; closed at 13.3 weeks over 26 weeks [66]. However, in a prospective study of using dHACM versus Apligraf for DFUs-dHACM had an astounding closure rate of 97% at 12 weeks, compared to 73% for Apligraf® and 51% for standard of care [67]. Therefore, whether you are interested in dHACM or not pretty clearly Apligraf®, out-performs standard of care for DFUs.

Dermagraft®

Dermagraft® (Organogenesis, Canton, MA) is a fibroblast derived dermal matrix. Fibroblasts from cryopreserved, allogenic, human, neonatal foreskin are cultured onto a bioabsorbable mesh. As they proliferate, they secrete collagen, matrix proteins, growth factors, and cytokines. The FDA has approved their use in the treatment of full-thickness diabetic foot ulcers of greater than 6 weeks duration that extend through the dermis, but without tendon, muscle, joint capsule, or bone exposure. It has been shown to stimulate cellular infiltration, angiogenesis, and epithelialization. Fibroblasts may result in better wound healing and less myofibroblastic activity. In addition cellular dermal substitutes may promote more rapid vascularization than their acellular counterparts. A randomized, controlled trial by Marston in 2003 demonstrated statistically significant improvement in closure rates when using Dermagraft® as compared to wet-dry dressing (30% to 18.3% at 12 weeks) for DFUs [68]. A randomized, controlled trial by Harding and colleagues (2013) showed a statistically significant benefit for only those with ulcers of less than 12 months duration [69]. The study design allowed for four treatments over an eight-week period (weeks 0, 1, 4, and 8). Dermagraft® works best on a maximally prepared wound beds. Another study by Hart et al. demonstrated that DFU patients managed with Dermagraft® based therapy alone healed significantly faster in comparison to patients treated with standard wound care therapy, with 30% closure compared to a 18.3% closure by the 12-week mark, respectively [70].

Micrografting

Micrografting has been described since the late nineteenth century, since Reverdin first described the technique of pinch grafting [71]. Multiple modifications ensued, including the stamp technique [72], the development of a microdermatome [73], intermingled allograft and autograft (Chih-Chun 1982) [74], minced autograft suspension [75], and even microskin sprays [76] have been proposed, however application to chronic wounds has been limited by poor cosmesis, the need for meticulous preparation, and limited efficacy compared to conventional meshed split thickness skin graft (STSG). Despite this, there has been renewed interest with the advent of two novel skin substitute systems: Recell® (Avita Medical, Melbourne UK) and Cellutome™ (KCI, San Antonio, TX). Recell® is a stand-alone system that allows for autologous cell harvesting, processing, and delivery. A small 1 cm × 1 cm STSG is obtained from the patient and is then processed with trypsin. This produces a population of keratinocytes, melanocytes, Langerhans cells, and fibroblasts which are then diluted in a lactate solution. The resulting solution of spray-on skin can be utilized to treat a wound as large as 80 cm^2 (1:80 ratio of STSG to wound surface area). The initial prospective trial on this novel treatment was published by Gravante in 2007 and demonstrated non-inferiority of Recell® when compared to those who received standard STSG [77]. It appears that Recell is undertaking trials specifically looking at their products effect on DFUs in both the UK and the USA. The trial is currently enrolling and looks like it will involve up to three ReCell treatments (initial treatment then additional treatments at 1 and 6 weeks if the doctor judges necessary). With the patients being followed out to 28 weeks.

Cellutome™ is an outpatient epidermal harvesting system (Cellutome™ KCI). Microdomes are harvested using gentle warmth and negative pressure, and are placed on the wound bed in the same session. Clinical trials are currently underway to evaluate its efficacy. This technology generally requires a maximally prepared wound bed prior to treatment. These epidermal bullae grafts have the interesting property of taking on their phenotype from their recipient site fibroblasts, therefore potentially allowing for skin that works more like plantar foot skin than a traditional skin graft [78]. To date a smattering of case studies and expert opinion pieces have looked at or discussed epidermal basal layer grafts, but no true study has been undertaken.

Engineered Products (Biomimetics)

Synthetic Skin Substitutes

Synthetic skin substitutes are highly processed and biologically inert combination wound coverings.

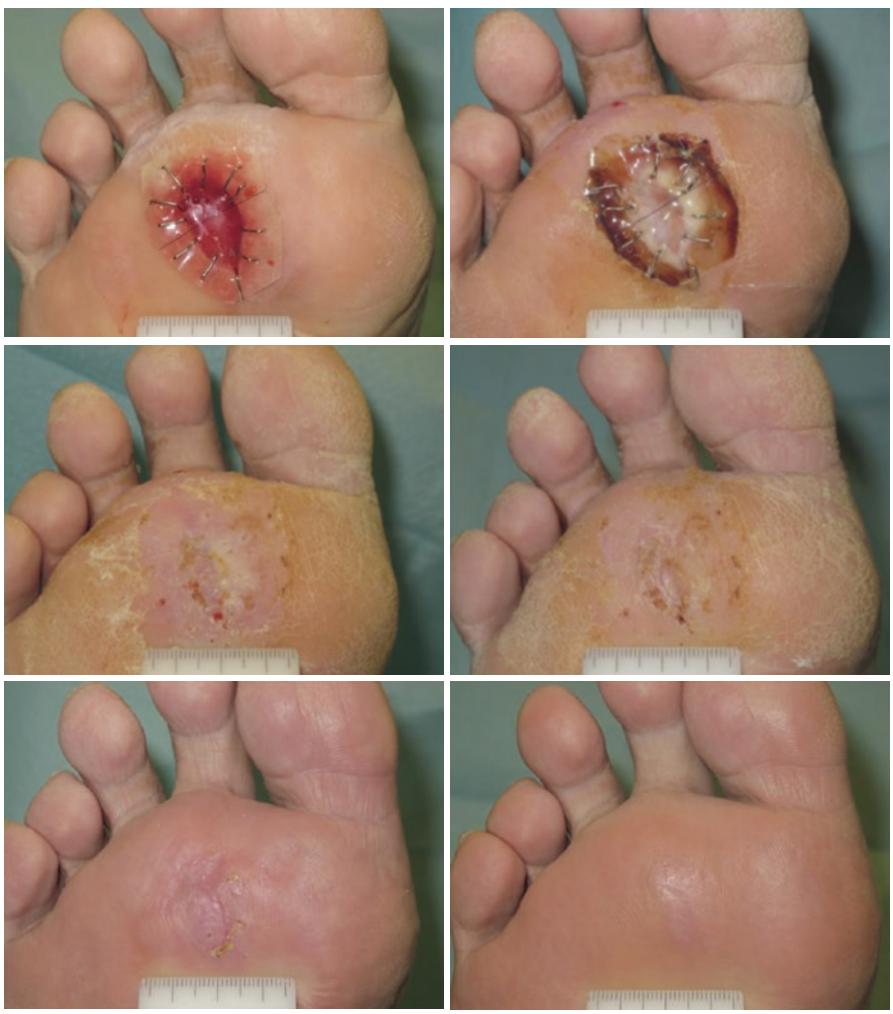
Biobrane®

Biobrane® (UDL Laboratories Inc., Rockford, IL) is a synthetic wound healing substitute developed from a layer of silicone bonded to a nylon membrane. It has been approved by the FDA for the treatment of clean partial-thickness burn wounds and donor site wounds. It has been investigated for nonhealing ulcers to promote a healthy granular wound bed. This product functions as a wound bed preparation agent itself and thus does not require thorough wound preparation prior to placement. It has not been studied in diabetic foot ulcers.

Integra (Omnigraft)®

Integra Matrix Wound Dressing® (Integra Life Sciences, Plainesboro, NJ) is composed of cross-linked bovine collagen and glycosaminoglycan (GAG) biodegradable matrix. The FDA has cleared Integra Matrix Wound Dressing® for its use in diabetic ulcers, partial- and full-thickness wounds, pressure ulcers, venous ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds, and draining wounds. This provides a scaffold for cellular invasion and capillary growth. Integra BiLayer Matrix Wound Dressing® (Integra Life Sciences Corp., Plansboro, NJ) Integra Dermal Regeneration Template (IDRT) has the same collagen GAG matrix with a semipermeable silicone membrane, which aids in water vapor loss, acts as a bacterial barrier, and increases tear strength. After adequate vascularization, usually requiring 2–3 weeks, an ultrathin epidermal autograft can usually be placed. The FOUNDERS trial was published in 2015, in which individuals with diabetes mellitus with Wagner Stage I and Stage II DFUs were treated with IDRT. This was a multicenter, randomized, controlled, parallel group clinical trial conducted under an Investigational Device Exemption. Thirty-two sites enrolled and randomized 307 subjects with at least one DFU. The subjects were randomized to the control treatment group (0.9% sodium chloride gel; $n = 153$) or the active treatment group (IDRT, $n = 154$). The treatment phase was 16 weeks or until confirmation of complete wound closure (100% reepithelialization of the wound surface), whichever occurred first. Complete DFU closure during the treatment phase was significantly greater with IDRT treatment (51%) than control treatment (32%; $p = 0.001$) at 16 weeks (Case 2). The median time to complete DFU closure was 43 days for IDRT subjects and 78 days for control subjects in wounds that healed. Of those that closed 72% required only one application of IDRT [79].The primary implication of this study in the author's mind is that not all DFU therapies need to be multiple application therapies.

Case 2 DFU treated with IDRT



Hyalomatrix®

Hyalomatrix® is a nonwoven pad comprised of an inner wound contact layer made of a fibrous form hyaluronic acid (HA) derivative and an outer layer comprised of a semipermeable silicone membrane. HA is present in skin, where it plays a major role in tissue repair, and in cartilage and connective tissue, where it functions as a lubricant and a component of the extracellular matrix. HA is present in the body as a polymer (a linked chain of many individual HA units), is continually produced by hyaluronan synthases, and is degraded naturally by a category of enzymes called hyaluronidases. This wound contact layer is biodegradable, and it acts as a 3D scaffold for cellular invasion and capillary growth. The silicone layer controls water vapor loss and provides protective coverage of the wound.

There has only been one prospective trial that included patients with DFU. This study was conducted in Italy and included 262 elderly patients from 70 centers. The patients were observed from that start of treatment with a dermal substitute (Hyalomatrix® PA [HPA]) until healthy dermal tissue suitable for a thin autograft was visible or until the growth of new epithelium from the wound edge was reported. Tracking wound edge advancement was used to assess the dermal substitute's performance. The main endpoint was the reduction in threshold area (10%) of the ulcer. Treated ulcers were characterized as follows: 46% vascular, 25% diabetic foot, 12% traumatic wounds, 2% pressure ulcers, and 15% other. Reepithelialization (^{3} 10%) was achieved in 83% of ulcers in a median time of 16 days [80]. Obviously, this is not very robust data.

Extracellular Matrix Products [ECM]

The extracellular matrix (ECM) is the largest component of the dermal skin layer and its disruption in chronic wounds is the hallmark of failed closure. The proteins contained in the ECM of normal skin are important in the healing of acute and chronic wounds. Without the proteoglycans and glycosaminoglycans of the ECM, the growth factors necessary for wound closure cannot work. The high levels of proteases found in chronic wounds impair healing by degrading essential components of the ECM [81–83]. Based on this theory, new dressings have been developed that are designed to reduce protease levels in wound fluids. This is done by providing a competitive substrate (collagen) for the proteases and thereby reducing proteolytic destruction of essential ECM components (fibronectin) and platelet-derived growth factors (PDGFs). However, the most common treatment for the corrupted ECM is replacing it from an exogenous source.

Xenograft ECMs

OASIS Wound Matrix® (Cook Biotech. Inc., West Lafayette, IN) is an intact, single layer, decellularized wound matrix derived from porcine small intestinal submucosa (SIS). The FDA has approved OASIS Wound Matrix® as a Class II (moderate risk) device. It is intended for single use in the management of various wounds such as those that are partial- and full-thickness, related to pressure, venous, diabetic, and chronic vascular ulcers, tunneled/undermined wounds, surgical wounds, trauma wounds, and draining wounds. OASIS Ultra® (Cook Biotech, Inc., West Lafayetter, IN) provides the same structural components, but is composed of three layers of ECM. The SIS wound matrix is thought to actively stimulate the proliferation of various cell types, enhance wound contraction, and help prevent catabolism by matrix metalloproteinases (MMPs) and matrix degrading enzymes. In 2015 Smith & Nephew (the distributor of Oasis) published a prospective DFU trial. Eighty-two subjects with DFUs (41 in each group) were assessed in the intent-to-treat analysis. Ulcers managed with SIS had a significantly greater proportion closed by 12 weeks than for the Control group (54 vs. 32%, $p = 0.021$) and this proportion was numerically higher at all visits. Time to closure for ulcers that healed was 2 weeks earlier for the SIS group compared with the standard care group. Median reduction in ulcer area was significantly greater for SIS at each weekly visit (all p values<0.05). As usual, these were Wagner 1 and 2 ulcers with good offloading.

PriMatrix™ (Integra, Plainsboro NJ) is an acellular ECM derived from fetal bovine dermis. The fetal collagen substrate contains a high proportion of type III collagen, which can bind growth factors while providing architecture to facilitate cell migration, proliferation, and differentiation. The FDA approved this product for the management of wounds, including partial- and full-thickness wounds, pressure, diabetic, and venous ulcers, second-degree burns, surgical wounds, trauma wounds, tunneled/undermined wounds, and draining wounds. In 2011, Karr and colleagues published a retrospective review demonstrating improved healing rates in DFU as compared to bilayer living cell therapy (Apligraf®, Organogenesis Inc., Canton, MA). The average number of applications in this study was 1.5 for DFUs (range 1–3 applications). In 2014 Kavros et al. published a prospective study of 55 DFU patients from the Mayo Clinic that showed 76% healing at 12 weeks, with an average time to heal of 7.5 weeks, and an average of 2.1 applications. Integra is opening a small prospective randomized FBC trial looking at DFUs in the end of 2016 to first quarter 2017 [84].

Endoform Dermal Template® (Mesynthes Ltd., Lower Hutt, New Zealand) is an intact, acellular extracellular matrix derived from bovine forestomach that has been used in multiple wound types. The FDA concluded that this product is similar to Integra™ and Oasis™. Indications for use include treatment of diabetic ulcers, partial- and full-thickness wounds, pressure ulcers, venous ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds, and draining wounds. This dressing, while functioning and

looking like a more expansive ECM dressing, demonstrated the ability to support cell attachment and differentiation while also inhibiting matrix metalloproteases and neutrophil elastase. It is actually categorized by the Centers for Medicare & Medicaid Services as a collagen (less expensive, more frequent applications) [84]. In 2012, Liden and colleagues performed a non-comparative study that demonstrated similar closure rates between Endoform® and other acellular dermal matrices. However, randomized, controlled trials on this product have yet to be published.

Kerecis is an acellular fish skin for medical use patented by Kerecis™. Like human skin, fish skin consists of cells embedded in a network of nonliving tissue. Gentle tissue preparation allows the proteins and lipids to remain in their natural state. This also allows for the preservation of omega 3 lipids. Of note is that no specific DFU trials have been performed. However, publication of a large prospective series that included many DFU patients is expected in the near future.

At the 2016 Asian Society of Vascular Surgery meeting, we presented cost effectiveness data on Kerecis in DFUs. We decided to use it as a way of seeing how one active extracellular matrix (ECM) therapy performed against expectations. If one was then to apply a standardized cost model across the two groups one could project potential cost savings. Data from 27 diabetic wounds treated with acellular fish skin, which was then inserted in a prognostic model [85], and compared with the actual outcome of the treatment. These 27 patients had a median 5.6 applications of fish skin; they took a median of 8.7 weeks to close and 89% closed. This closure rate was then compared to Dr. Margolis prognostic DFU model that stated that a 42% would close after 20 weeks. This delta between the two groups was then multiplied against a standardized cost model that includes the cost of product, the cost of application, and weekly versus daily standard of care dressing and nursing costs. The fish skin treated patients healed 112% more often than predicted at 20 weeks. This resulted in a 62.5% cost reduction for those patients that healed [86].

Allograft ECMs

Acellular dermal matrices are used in a variety of reconstructive and cosmetic procedures. There seems to be host tissue integration, revascularization, and recellularization into these products, but the exact timing and differences among them remain unknown [87–89]. A study was actually undertaken to determine and compare these properties of four different acellular dermal matrices (AlloDerm, DermACELL, DermaMatrix, and Integra) in an in vivo rat model. Tissue specimens were obtained at various time points. Histology and immunohistologic assays were used to quantify the extent of cellular infiltration and revascularization within the various matrices. A bimodal cellular response was observed in all products except for DermACELL [90, 91]. Cellular infiltration was highest in DermACELL and lowest in AlloDerm, and angiogenesis was evident by day 7. There were clear differences within the various products. It is undetermined whether these differences are advantageous or clinically significant [92, 93]. Future work is needed to define the specific roles for each.

Alloderm® (LifeCell Corp., Bridgewater, NJ, which is actually held by the same parent company as KCI, San Antonio, TX), is derived from acellular cadaveric dermis. It is an allogenic skin substitute most commonly used in the repair of complex abdominal wall hernias [94, 95]. AlloDerm® is classified as human tissue and subject to the rules and regulations of banked tissues, regulated by the American Association of Tissue Banks (AATB) [96, 97]; hence, it is not subject to FDA prenotification approval. The manufacturing of this matrix begins with donated human skin that is then cleared of the cellular components that are responsible for an immunogenic response [98, 99]. While this product has not been seen in real time use in the diabetic foot is can be used in the operating room and should be considered to act similarly to its cousin [100–102].

GraftJacket® (KCI, San Antonio, TX) is an acellular scaffold originating from human cadaveric tissue that has demonstrated usefulness mainly in diabetic skin ulcers and orthopedic soft tissue injuries [103–105]. This is for all intents the same product as Alloderm®. Winters et al. published a multicenter, retrospective study of acellular dermal regenerative tissue matrix as an alternative treatment for 100 chronic, full-thickness wounds of the lower extremity in 75 diabetic patients. Some of these were pretty advanced on the University of Texas (UT) wound classification, (34.0%) 3D. The mean time to matrix incorporation, 100% granulation, and complete healing was 1.5 weeks (0.43–4.4 weeks), 5.1 weeks (0.43–16.7 weeks), and 13.8 weeks (1.7–57.8 weeks), respectively. The overall matrix success rate, as defined by full epithelialization, was 90.0%. Absence of matrix-related complications and high rates of closure in a wide array of diabetic wounds suggest that this matrix is a viable treatment for complex lower-extremity wounds. Lack of any statistically significant differences between UT grades and wound outcome end points lends further support to the universal applicability of this matrix, with successful results in both superficial diabetic wounds and in wounds penetrating to the bone or joint [106, 107].

In a randomized, controlled trial, patients with diabetic foot ulcers (DFUs) were standardized to receiver either treatment with GraftJacket® or the standard of care therapy for DFUs at that institution. These authors demonstrated that patients treated with GraftJacket® experienced significantly better healing time as well as a significantly lower nonhealing rate as compared to controls. Of note, this product works

best on a highly prepared wound bed. To this end Brigido performed a prospective, randomized, controlled study to evaluate the efficacy of sharp debridement plus Graftjacket application versus sharp debridement only in 28 patients with diabetes over a 16-week period. Results demonstrate complete healing by week 16 in 12 out of 14 patients in the Graftjacket group versus four out of 14 complete wound closures in the sharp debridement only group. The author concluded that the use of Graftjacket in addition to sharp debridement can lead to a statistically significant increased percentage of complete healing of lower-extremity ulcerations [108].

Reyzelman and coworkers conducted a prospective, randomized, controlled, multicenter study comparing 47 patients treated with one Graftjacket application versus 39 patients receiving standard care (moist wound therapy using alginates, foams and hydrogels as per the physician's discretion) [109]. They noted complete healing in 69.6% of the Graftjacket group and 46.2% of the standard care group. The average time to healing was 5–7 weeks in the Graftjacket group and 6–8 weeks in the standard care group. These results demonstrate that DFUs treated with Graftjacket were two to three times more likely to heal than DFUs treated with standard wound care therapy alone [110, 111]. Overall, it appears that there evidence for the enhanced closure of DFUs with this product and, in our opinion, it is surprising that this product has not enjoyed wider acceptance.

While other Decellurized-Acellular Dermal Matrixes (D-ADMs) have been clinically available, the one assessed in this study represents new treatment technologies. The patented process used to prepare this specific D-ADM includes the use of anionic detergents and an endonuclease resulting in a material with more than 97% nucleic acid removal and acellular histological appearance. In addition, this new graft is preserved and stored at room temperature, allowing the allograft to be maintained and delivered fully hydrated and at ambient temperature to the surgical suite or outpatient procedure room. Other dermal allografts must be shipped and stored frozen at subzero temperatures or freeze-dried, requiring solvent rehydration before implantation [112].

DermACELL recently reported on a 168 patient study versus a standard of care and a Graftjacket arm. One hundred sixty-eight patients were randomized into DermACELL, conventional care, and Graftjacket treatment arms in a 2:2:1 ratio [113]. Patients in the acellular dermal matrix groups received either 1 or 2 applications of the graft at the discretion of the investigator. At 16 weeks, the DermACELL arm had a significantly higher proportion of completely healed ulcers than the conventional care arm (67.9 vs. 48.1%; $P = 0.0385$) and a no significantly higher proportion than the Graftjacket arm (67.9 vs. 47.8%; $P = 0.1149$). Overall this appears to support the use of ADMs over standard of care.

Similar dermal xenografts as alternatives populate the market as well. Acellular dermal xenografts are often chemically cross-linked, theoretically making them less suitable for wound healing. Products in this group are Permacol (Tissue Science Laboratories, Hampshire, UK), a porcine-derived acellular dermal matrix, and EZ-Derm (Mölnlycke Health Care AB, Gothenburg, Sweden), a collagen matrix derived from porcine dermis [114, 115]. The use of Permacol as a dermal substitute for wound healing purposes has indeed largely been abandoned, and clinical results of EZ-Derm in wound healing are not convincing [116, 117].

Amniotic Products

Let it first be acknowledged that the proprietary nature of the CTP business detracts in some ways from patient care. Each company has a claim that their proprietary product works better than another. Most of these claims lay in the processing component of the material. The authors will present to the reader comparison trials when available. In general these products as a whole probably work by bring precursor cells to the wound bed and by delivering growth factors. These tissues in general are rich in cytokines and growth factors known to promote wound healing; however, preservation of the biological activities of therapeutic allografts during processing remains a challenge. Various companies have made claims around the presence of growth factors: platelet-derived growth factor-AA (PDGF-AA), PDGF-BB, transforming growth factor α (TGFα), TGFβ1, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), placental growth factor (PLGF), and granulocyte colony-stimulating factor (GCSF).

EpiFix® (MiMedx Group, Inc., Marietta, GA) is engineered from human allograft amniotic membrane, composed of an epithelial layer, a basement membrane, and a connective tissue matrix. Epifix® is regulated by the FDA as Human Cells, Tissues, and Cellular and Tissue Based Products. Indications include wound management for patients with neuropathic DFUs. It is believed that the collagen and extracellular matrix provided by the amniotic membrane function to promote cellular proliferation and delivery of growth factors thus facilitating cellular ingrowth. A recent multi center, prospective, randomized, comparative study of 60 patients was conducted. The primary study outcome was the percent change in complete wound healing after 4 and 6 weeks of treatment. Secondary outcomes included percent change in wound area per week, velocity of wound closure and a calculation of the amount and cost of Apligraf or EpiFix used. A total of 65 subjects entered the 2-week run-in period and 60 were randomized (20 per group). The proportion of patients

in the EpiFix group achieving complete wound closure within 4 and 6 weeks was 85 and 95%, significantly higher (all adjusted *P*-values ≤0.003) than for patients receiving Apligraf (35 and 45%), or standard care (30 and 35%). After 1 week, wounds treated with EpiFix had reduced in area by 83.5% compared with 53.1% for wounds treated with Apligraf. Median time to healing was significantly faster (all adjusted *P*-values ≤0.001) with EpiFix (13 days) compared to Apligraf (49 days) or standard care (49 days). The mean number of grafts used and the graft cost per patient were lower in the EpiFix group compared to the Apligraf group, at 2.15 grafts at a cost of $1669 versus 6.2 grafts at a cost of $9216, respectively [118].

While this product is marketed as being useful in many wound types, however, the above study demonstrates specific its utility in diabetic foot ulcers. A recently published interim analysis of a three-armed trial showed improved healing rates versus standard of care and Apligraf. The proportion of wounds achieving complete closure within the 12-week study period were 73% (24/33), 97% (31/32), and 51% (18/35) for Apligraf, EpiFix and SWC, respectively (adjusted $P = 0.00019$). Subjects treated with EpiFix had a very significant higher probability of their wounds healing [hazard ratio (HR: 5·66; adjusted P: $1{\cdot}3 \times 10(-7)$] compared to SWC alone [119].

Grafix® is a human viable wound matrix (hVWM) that the company claims is manufactured utilizing a novel technology (cryopreservation) that enables the preservation of all placental membrane components in their native state. They believe that this preserves an ECM component but more importantly mesenchymal stem cells. In 2014 they published a prospective randomized trial ($N = 50$), to standard wound care ($n = 47$) for DFU healing. The primary endpoint was the proportion of patients with complete wound closure by 12 weeks. Secondary endpoints included the time to wound closure, adverse events and wound closure in the crossover phase. The proportion of patients who achieved complete wound closure was significantly higher in patients who received Grafix (62%) compared with controls (21%, $P = 0.0001$). The median time to healing was 42 days in Grafix patients compared with 69.5 days in controls ($P = 0.019$). Treatment with Grafix significantly improved DFU healing compared with standard wound therapy. Importantly, Grafix also reduced DFU-related complications. The results of this well-controlled study showed that Grafix is a safe and more effective therapy for treating DFUs than standard wound therapy. The company has subsequently supported a study showing efficacy over tendon and bone; however there was no control arm in that study and the wound appears to have been post surgical [120]. It would have been a stronger study if compared to NPWT.

Amniox (AmnioBand, Musculoskeletal Transplant Foundation, Edison, NJ) is an aseptically processed dehydrated human amnion and chorion allograft (dHACA). There is a lot of discussion in this space as to whether the chorion is beneficial or not. This product has been studied for the treatment of DFUs in a 40 patient prospective trial. Patients with DFUs treated with standard of care (SOC) (offloading, appropriate debridement, and moist wound care) after a 2-week screening period were randomized to either SOC or wound-size-specific dHACA applied weekly for up to 12 weeks plus SOC. At 6 weeks, 70% (14/20) of the dHACA-treated DFUs healed compared with 15% (3/20) treated with SOC alone. Furthermore, at 12 weeks, 85% (17/20) of the DFUs in the dHACA group healed compared with 25% (5/20) in the SOC group, with a corresponding mean time to heal of 36 and 70 days, respectively. The mean cost to heal the wounds that healed with the dHACA was $1400. This was significantly cheaper than some of the other DFU CTP models [121].

Biovance (Celgene Cellular Therapeutics, Morris, New Jersey) is a wound covering produced from decellularized, dehydrated human amniotic membrane. A study of 14 patients with DFUs was undertaken. The purpose of this study was to determine healing rates for partial- and full-thickness diabetic foot ulcers treated with Biovance. The secondary objective was to determine time to complete wound closure and safety profile. Groups 1 and 2 (55.5% and 33.3%, respectively, comprising 60.1% of total participants) received a benefit from using Biovance wound covering, and there were no adverse reactions to the tissue. The company started a prospective randomized trial and started enrolling patients, but has closed the study. It is not clear if they will attempt to publish their partial data set.

Negative Pressure Wound Therapy (NPWT)

NPWT refers to the suction applied to a wound bed through foam, gauze or an engineered dressing. These dressing are occlusive and in most cases, whether the device is mechanical, electric or single use disposable, they appear to work in similar fashion. Larger units with larger canisters are necessary for larger, deeper, and high drainage wounds. The negative pressure units can generate negative pressure at 80–200 mmHg depending upon their engineering. These units appear to work by macrodeformation and microdeformation which pull the wounds together, increase vascularity and decrease wound edema while increasing lymphatic drainage through an unclear mechanism [122].

A number of studies have compared the use of NPWT to standard wound care. In a multicenter, randomized-controlled trial with 342 patients, Blume et al. compared NPWT to wounds treated with alginate or hydrogel dressings in DFUs.

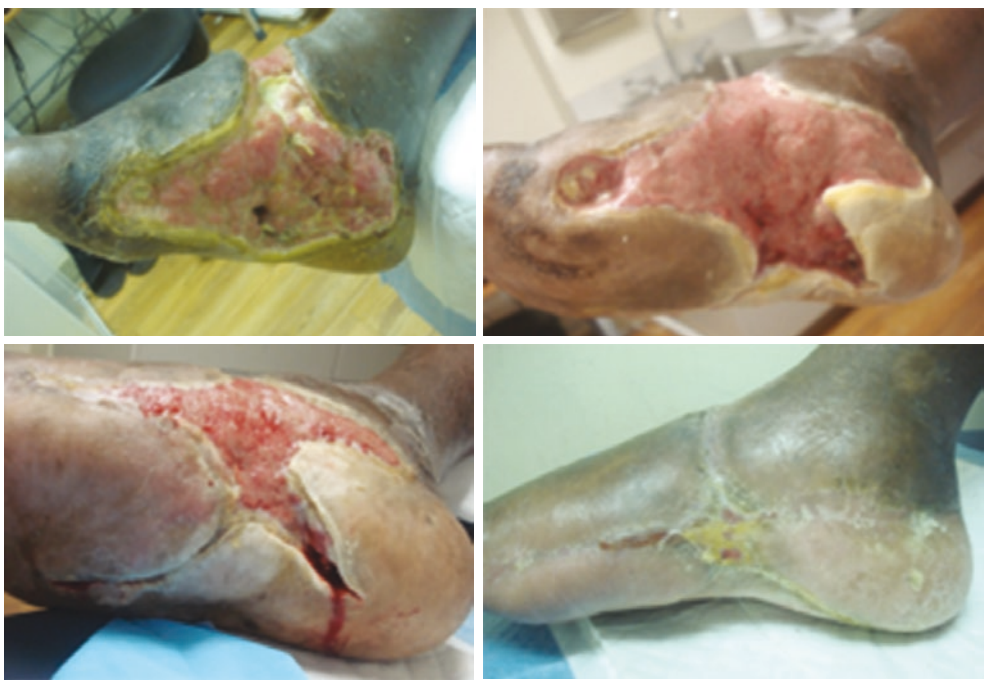
The authors found higher wound closure rates in ulcers randomized to NPWT treatment and concluded that NPWT is a safe and effective modality for improving the healing potential of diabetic foot ulcers [123]. However, several limitations that could have altered the outcome have been noted including a high dropout rate (only 68% of patients completed the study), non-blinding, and failure to standardize ancillary treatments such as use of antibiotics, offloading, and intermittent versus continuous negative pressure methods. A post hoc analysis of these data showed a median cost per cm reduction benefit $1106 in favor of NPWT; this metric seems to be a good one if costs can be standardized in the model [124].

NPWT has also been used following partial foot amputations with reported success. In this randomized, multicenter study, 162 patients with open wounds following partial foot amputations at the metatarsal level were treated with NPWT or standard moist wound dressings [125]. The authors reported NPWT treated wounds healed more frequently, at a faster rate, and formed granulation tissue more rapidly compared to the standard wound care group. They concluded that NPWT treatment was a safe and effective method for accelerating the rate of wound closure and had the potential to reduce reamputation rates. While this report demonstrated the promise of NPWT treated wounds following partial foot amputations, the rate of wound closure was improved in the NPWT group only when surgical wound closure was included in the analysis. The decision for surgical wound closure was not clearly defined or described in the study, potentially limiting the support for the effectiveness of the NPWT as a stand-alone treatment.

As with all new modalities, the cost of NPWT is of critical importance in determining its role. Based on the data from the previous study, Apelqvist et al. performed a cost analysis based on length of hospital stay, procedures performed, and number of dressings changed on the 162 patients [126]. The authors concluded that a savings of $12,800 was realized when NPWT was used as a result of diminished resource utilization such as fewer physician visits and wound care dressings needed. Furthermore, the patients treated with NPWT experienced higher rates of wound healing, also impacting the length of care needed.

While the role of NPWT in the care of diabetic foot ulcers remains a source of considerable debate, most systematic reviews and consensus statements have supported its ability to improve the healing process. Certainly after significant foot reconstruction, NPWT has changed the way lower-extremity foot reconstruction is handled (Case 3). In addition, good wound care including periodic, aggressive debridement, pressure offloading, as well as concomitant use of active wound care dressings such as acellular matrix scaffolds was encouraged in combination with NPWT.

Case 3 Limb salvage with NPWT



Comments on Diabetic Foot Ulcer Studies

Currently there are 482 studies concerning DFU listed on ClinicalTrials.gov. Of these 128 are open. MEDILNE and ClincalTrials.gov literature searches identified 291 publications, including 80 citations addressing 41 RCTs on effects of topical agents applied to a DFU.

However, the question of translational relevance remains. Typically, years of preclinical research precede a clinical trial. Yet with many wound care products, this has not been the case as companies race to bring new products to market. This has left the field with a large number of similar or "me-too" products. Only well-designed, well-conducted, prospective double-blind RCTs can determine whether an agent works safely and improves clinical outcomes.

Five systematic reviews have described common flaws or issues observed in relevant RCTs. Most trials include an adequate sample size for Phase II studies of at least 20 subjects per group. However, very few (basically only the PMA trials) include enough patients for a pivotal Phase III trial. These sample sizes requires estimates that are sufficient to achieve statistical power of at least 80% with a 5% Type 1 (alpha) error of falsely concluding healing efficacy (statistically rejecting the null hypothesis) for a clinically important difference in the primary outcome, for example a 15% difference in 12-week healing rates between treated and control subjects. For new products, regulatory authorities require a two-tailed Type 1 error in this calculation, assuming equal likelihood of benefit or harm of the active agent in Phase III studies, inflating the sample sizes required. While this may seem absurd if healing outcome(s) significantly favored the active agent in its Phase II studies, the inflated sample sizes help assure that related safety issues are addressed on the package insert.

Very few trials in the CTP area, and the entire wound care space, include randomized allocation to groups without bias. Using unbiased group allocation at the point of subject assignment is required to validate superiority of one strategy over another. Blinding can be extremely difficult in these studies, but at minimum the evaluation must be done in a blinded manner. Most trials do not meet these criteria and therefore have significant bias.

When blinding is not feasible, the reasons should be clearly described in all public communications. Inclusion and exclusion criteria need to be clearly described, as well as the length of the run-in phase. Using those inclusion criteria known to predict healing to stratify group assignment can help avoid imbalanced groups at the time of analysis. Alternatively, baseline inclusion measurements can support planned analyses that clarify treatment effects independently of baseline group differences.

One very signifciant failure of trials for DFU in that only patients that have not experienced healing of at least 50% at 4 weeks should be included in trials. This is because approximately 50% of ulcers are healed by 12 weeks with standard of treatments, and as such many patients may not need the particular intervention being evaluated. Including only those DFUs that reduce in surface area less than 50% with standard treatment for 4 weeks (or less than 25–30% during a pre-randomization 2-week screening period) focuses on those that are unlikely to heal with continues standard therapies during the next 12–20 weeks.

As mentioned previously, most studies only include Wagner 1 and 2 ulcer grade patients, however, baseline ulcer depth is an equally important predictor of healing that should be considered in developing inclusion criteria. Standardized Wagner or University of Texas grading scales confound DFU depth with abscess, infection, and/or ischemia. Neither of these DFU grading systems clearly differentiate full- from partial-thickness DFUs, which differ significantly in their healing processes. Generally, a deeper wound requires more granulation tissue before it can begin to reepithelialize. It has been documented that full-thickness ulcers require twice the time to heal as similar sized partial-thickness ulcers in the same cohort. It should be recognized that depth is difficult to assess and hopefully some of the new imaging technologies will make this arduous task easier. Likewise the frequency and extent of debridement need to be standardized across study groups. One fetal bovine dermis study currently underway is attemptig to do this.

Outcome measures also need to be standardized across all studies, as do economic measures. Two healing outcomes recognized by the United States Food and Drug Administration (FDA) are (1) time to complete healing of the study DFU with no visible wound or residue, verified as completely closed 2 weeks later and (2) percent of study DFU completely healed, similarly verified 2 weeks after initial healing, following a standardized interval between first treatment and outcome measurement, usually of 10 or 12 weeks.We believe that recognition of percent area reduction as an endpoint, would allow for a greater number of synergistic studies, and that cost per square centimeter area reduction is a good comparative indicator that should be a standardized requirement of all studies [124]. Using pedictive models of expected healing rates and applying them to study populations, in order to see that they conform, also would appear to be a way to standardize many of the biases currently noted in studies [85, 86]. Finally, recurrence needs to be assessed therefore, all studies should require a 6 month and 1 year recidivism survey.

If the above guidelines were adhered to, better cost decisions and algorithm of care guidelines could be advanced. As Dr. Bolton stated elegantly; "design, conduct, and reporting to generate quality, credible wound care evidence that propels promising DFU healing agents forward. This high-quality research has the potential to generate well-researched breakthroughs in DFU management. Global patients, clinicians, payers, product developers, and governments all gain

if wound care researchers routinely apply these rigorous principles of RCT quality" [127]. Clearly patients, clinicians, and reimbursement authorities deserve stronger science to inform their decisions.

If we apply the above criteria to the published literature we see the 41 unique RCTs that reported recognized DFU healing outcomes, couldn't be compared. This is due to; variations in SOC, (e.g., offloading varying from crutches or wheelchairs to walker boots held in place with bandaging), length of follow-up (none to 12 weeks) and subject baseline characteristics (e.g., duration of diabetes and degree of metabolic control) and DFU inclusion criteria (ulcer duration, depth, and area on enrollment, healing or nonhealing status, Wagner or University of Texas Grade 1 or 1–2). Fifty-one percent 21/41 were double-blind studies, with six more (15%) blinded to either patient or outcome evaluator. Nearly half of these trials had high potential for bias because those evaluating results knew which treatment each subject received. In the future the WiFI method of rading these ulcers may be a much better option, but to date no studies have employed this grading system.

In short, studies based on standard guidelines that build upon previous work should be the norm. However, such studies are very difficult to fund, and execute. In addition the differing pathways to market in the United States make it sensible for commercial entities to pursue less than adequate clinical research. Of note is that it has recently become apparent that payers, including large insurance companies, are starting to require comparative efficacy studies for wound care products. In our opinion, this may represent a small step in the right direction. In addition synergistic combination studies that use algorithms of care that might employ what many companies would consider "competing" products need to be undertaken. Some of the algorithms may disenfranchise cerrtain groups that now provide wound care, and are therefore not commercially attractive.

Treatment According to Ulcer Stages

FDA-approved DFU products—those having gone through the premarket approval (PMA) process and acquired a specific indication—are indicated for Wagner 1 and 2 ulcers as those with grades 3 and above were excluded from clinical trials. Wagner 3 ulcers require drainage and bone resection, possibly with long-term antibiotic therapy, unless the bone margins are negative. Because premarket approval studies have not allowed for inclusion of wounds with exposed tendon, bone, or joint capsule, many extracellular matrix products and some amniotic products are routinely use to cover exposed structures, as they are FDA cleared. The distinction between FDA indication and clearance does have a bearing on reimbursement. As such it makes sense to try and use products more focused at dermal (re)-generation for the deeper wounds and those with exposed bone. Many studies have shown efficacy of products in covering these structures, but there is unfortunately no clear-cut consensus exists [128, 129]. Wagner 4 and 5 ulcers are usually treated with surgical resection and often covered with a NPWT device.

Conclusions

We believe that one fallacy in the treatment of diabetic foot wounds is that each patient's ulcer requires individualized care. This, we feel, is related to a fundamental lack of scientific rigor, and the fact that numerous entities and interests are involved in promoting their products. In a rational delivery system, diabetic foot wounds should be treated initially much like one treats cancer, using a standardized algorithm that works for a majority of cases. Deviations from these standards should be the subject of clinical trials. That there are so many products on the market for the care diabetic foot wounds speaks to the fact that we have not defined an appropriate standard of care. When reviewing diabetic foot wound literature, the absence of studies on patients with heel ulcers, those on renal replacement therapy, with HIV, hepatitis, or without an ideal vascular supply is glaring. Effectiveness trials that include real world populations therefore should be mandatory in the design of new studies.

Based upon available literature, it is appropriate to recommend that all patients with diabetic foot ulcers be treated with maximal offloading, most likely with a total contact cast, and with weekly debridement. One could also argue, potentially, for a topical foam dressing over one with antimicrobials for 4 weeks. If the wound has not reduced in size by 50% at 4 weeks of total contact casting care then wounds larger than 2 cm^2 should be widely debrided and treated with an acellular, tissue-based therapy in which case we would advocate for a extracellular matrix xenograph product as on the whole they appear to be more cost effective, than CTPs in the other product categories and most likely we would beleive that based on minimal but compelling data that products in his category that require fewer applications are equal but less expensive than ones that require more frequent applications. For wounds that are on the heel, or the toe, or that are less than 2 cm^2 one could pursue a topical growth factor strategy as these wound sites have not been included in cellular and tissue-based therapy trials. MMP reducing dressings or thin ECMs may have a role in conjunction with GFs which have not been thoroughly explored. Based upon basic science data the role for ECMs in combination with active growth factor therapy deserves more evaluation, while this may be how the amniotic products work and may support their current widespread use.

Finally, after major debridement or open resections of larger foot wounds, negative pressure wound therapy appears to be the most effective therapeutic option, and it may be applied over dermal regenerating CTPs with an eye towards preserving long-term function. It is the authors' hope that strict standards of care for this population's will become the norm, such as we employ for breast and colon cancer, that is based on Stage of the wound, and co-morbidity of the patient, and reimbursement and tolerance for treatment outside the standard of care will not be available unless on a research protocol.

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Surgical Treatment of the Ulcerated Foot

19

John M. Giurini

Abstract

Foot ulceration with infection continues to be one of the leading causes of hospitalization for patients with diabetes mellitus. The lifetime incidence of foot ulcerations may be as high as 25%. In spite of advances in the care of the diabetic foot, the rate of recidivism remains a staggering 50% with the majority of these ulcerations recurring within 18 months. This has significant economic ramifications on the healthcare system when one considers that the average total cost of healing an infected ulceration not requiring amputation is approximately $17,500 per episode.

Successful treatment of diabetic foot infections and ulcerations requires a thorough understanding of the risk factors for ulcerations and amputations. It also requires taking advantage of advances in antimicrobial therapy, advance wound healing strategies including topical growth factors, negative pressure wound therapy (NPWT), improved vascular interventions. An aggressive surgical approach should be considered when structural deformities contribute to the recurrence of ulcerations and when conservative management fails. The key to successful surgery is understanding the risks and foot mechanics and structure. Key components for successful outcomes require the establishment of treatment algorithms utilizing the advanced wound healing strategies, vascular intervention, and foot surgery. This requires the establishment of a dedicated team of healthcare professionals to manage these complex problems.

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Introduction

Foot ulceration with subsequent infection is one of the leading causes of hospitalization for patients with diabetes mellitus. The lifetime incidence of foot ulceration may be as high as 25% [1]. The rate of recidivism is also staggering in this population with 50% of ulcerations recurring within 18 months [2]. The number of lower extremity amputations among diabetic patients has been well documented for years. Approximately 73,000 nontraumatic lower-limb amputations are performed annually in people with diabetes [3]. More importantly, 85% of these amputations are preceded by a foot ulcer [4, 5].

The causative factors leading to ulceration are well documented with peripheral neuropathy being present in over 50% of patients [6]. The second causative factor that plays a significant role in diabetic foot ulcerations is excessive plantar pressure from limited joint mobility and foot deformities [1]. Current algorithms for treatment take advantage of recent advances in antimicrobial therapy and wound healing strategies including topical growth factor and negative pressure wound therapy (NPWT). However surgical intervention plays an increasingly more important role not only in the treatment of ulcerations but also in prevention of recurrent ulcerations. One of the key components in establishing successful outcomes is identifying a dedicated team of healthcare professionals who understand the role of surgery in managing these complex problems [7–10].

Goals of Surgery

It is important that the surgeon and patient realize that the goals of surgery in patients with neuropathy and history of ulcerations differ from the goals in patients with normal sensation and foot deformities. The surgeon must clearly delineate these goals to the patient and the patient must clearly understand the goals.

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Table 19.1 Surgical goals in the insensate patient

• Reduce risk for ulceration/amputation
• Reduce foot deformity
• Provide stable foot for ambulation
• Reduce pain
• Improve appearance of foot

The primary reason for surgical intervention in patients with normal sensation is to eliminate a patient's source of pain from an underlying deformity. Cosmesis is a secondary goal in this patient population. In diabetic patients with a history of a recurrent ulceration or with a current ulceration, the primary goal of surgery is to reduce the risk of lower extremity amputation by correcting a structural deformity leading to the ulceration, eliminating a site of high plantar pressure or eliminating a focus of osteomyelitis (Table 19.1). In this group of patients, cosmesis, while desirable, becomes secondary to the overall goal of limb salvage.

It is also important to distinguish between elective surgery, prophylactic surgery, and urgent surgery as it relates to diabetic foot. Elective surgery implies the presence of a deformity that does not put the patient or limb at immediate risk but may be corrected surgically. These type of deformities can be managed without surgery and there are clinical situations where this conservative approach is in the patient's best interest.

Prophylactic surgery is defined as surgery performed to prevent a more serious event, namely imminent amputation. In this case there is a history of a chronically recurrent ulceration with an underlying deformity that puts the limb at risk. Surgery in this scenario may be considered to correct the deformity and reduce the risk of recurrence and amputation.

Urgent surgery is self-explanatory. These patients will commonly present with foul-smelling ulcerations with purulent drainage and cellulitis. Necrosis and abscess formation are not uncommon. They may also have clinical signs of sepsis, e.g., fever, chills, and hypotension. These patients require immediate surgical intervention. The immediate goal of surgery in this situation is to control the infection, to stabilize the patient, and to save as much of the foot and/or leg as possible. In the majority of cases, these patients will require additional surgery to provide the patient with a functional foot and extremity.

Preoperative Evaluation

A detailed medical history, surgical history, list of current medications, and identification of risk factors such as smoking and nephropathy are critical to proper preoperative risk assessment. An assessment of the patient's diabetes control via HgbA1C can alert the surgeon to potential complications postoperatively. It has long been believed that poor metabolic control is associated with higher postoperative infection rates, delayed wound healing, and higher nonunion rates than in well-controlled diabetic patients or nondiabetic patients. This is believed to be secondary to the inhibition of leukocyte migration by elevated glucose levels. Recent reports show that random blood sugars >200 mg/dL were associated with increased rates of surgical site infections (SSI) [11]. Wound healing complications following foot and ankle surgery increased by a factor of 1.59 for every 1% elevation in HgbA1C [12]. Preoperative consultation with the patient's endocrinologist is warranted in order to optimize blood sugar management before surgery to minimize risks of complications, especially when elective or prophylactic surgery is contemplated. Optimal glycemic control may be achievable for elective or prophylactic procedures. However urgent or emergent surgery should not be delayed in order to achieve optimum control.

In addition to diabetes control, it is also important to assess patients for other complications of diabetes such as cardiac and renal complications. Both of these complications will influence choice of anesthesia as well as use of medications postoperatively. Because of autonomic neuropathy, the risk of silent myocardial infarction is real. In a recent study looking for subclinical myocardial damage, troponin levels were measured in nondiabetic, prediabetic, and diabetic patients at baseline and 6 years later [13]. Troponin levels were found to be significantly elevated after 6 years in prediabetic and diabetic patients over nondiabetic patients suggesting that subclinical myocardial damage had occurred in the absence of symptoms secondary to long-standing hyperglycemia. The authors concluded that those two groups were at substantially higher risk of heart failure, death, and coronary heart disease over nondiabetic counterparts. Therefore consultations with endocrinology, cardiology, and nephrology can be very valuable.

The vascular evaluation of the diabetic foot requires special attention and discussion. While the majority of diabetic patients with strongly palpable pedal pulses will usually heal a local foot procedure without difficulty, there are reports and instances of diabetic patients with palpable pulses and ischemic lesions [14, 15]. Patients with weakly palpable or nonpalpable pedal pulses require further vascular evaluation or a formal vascular surgery consultation. The ankle-brachial index (ABI) remains the most common method of diagnosing peripheral arterial disease in patients with diabetes [16]. However, the ABI can be falsely elevated due to arterial medial calcinosis and noncompressible vessels. Because digital vessels are less susceptible to medial artery calcinosis, toe-brachial index (TBI) as measured at the level of the great toe is considered more sensitive than ABI in diagnosing ischemia [17, 18]. Vascular intervention may often be necessary prior to any limb-sparing surgery [19, 20].

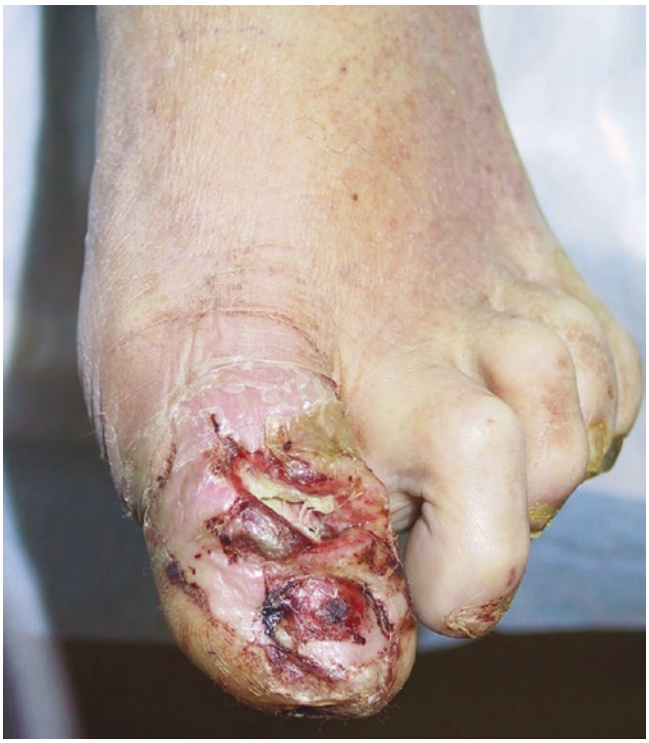

Fig. 19.1 Failure to recognize critical ischemia resulted in surgical failure in diabetic patient with autonomic neuropathy

Patients with autonomic neuropathy require special mention. These patients will often present with pink, warm skin on the surface of the foot. This can be easily mistaken for a foot with good arterial perfusion even in the presence of critical ischemia (Fig. 19.1). Skin temperature alone cannot be relied upon as a sensitive indicator of good perfusion.

Finally, a detailed social history has become increasingly important. More of the burden for the patient's aftercare is being placed on the patient's family. The majority of patients will require daily dressing changes and prolonged periods of non-weight-bearing. For this reason, visiting nurses, home health aides, and physical therapists have become vital members of the multidisciplinary team. In situations where there is less than adequate support for these services at home, admission to a rehabilitative center should be considered. These factors should be identified early in the course of the patient's hospitalization so that discharge planning can proceed in a timely and stress-free manner.

Anesthesia Techniques

The presence of profound peripheral sensory neuropathy and the localized nature of many of these procedures make local anesthesia with monitored intravenous sedation ideal for diabetic patients undergoing foot surgery. Epidural or general anesthesia should only be considered when more extensive surgery is being planned or for longer procedures when it is critical that patients remain immobile. This includes most major procedures of the hindfoot and ankle as occurs in reconstruction of the Charcot foot. It should be remembered that either of these techniques increases the perioperative morbidity and mortality. Therefore, the final choice of anesthesia should be made following discussion with the anesthesiologist and the patient's primary medical doctor and with a clear understanding of the procedure being performed.

Surgical Approach

Prior to definitive surgery or correction of an underlying deformity, the foot must be free of any acute infection. This implies that any area of undrained sepsis has been adequately drained and all necrotic tissue debrided to healthy granular tissue. The proper technique for draining wounds is to incise the wound in such a fashion to promote dependent drainage. As the patient lies recumbent in bed with the extremity elevated, the wound will drain from distal to proximal (Fig. 19.2) [21]. Multiple stab incisions with the use of Penrose drains should be avoided as they do not promote dependent drainage. Any tissue that appears infected or necrotic should be sharply excised at this time, including any exposed or infected bone. The wound is then packed widely open and inspected daily for the resolution of sepsis, cellulitis, and the

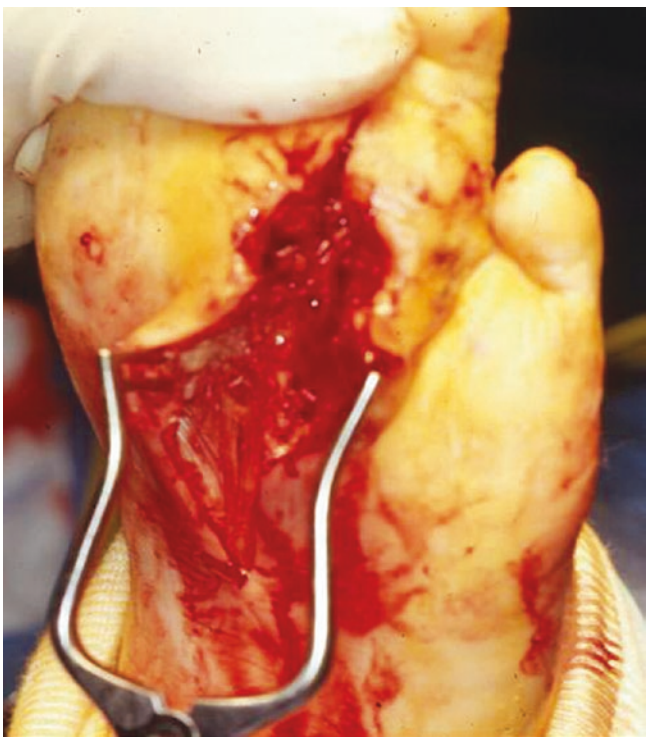

Fig. 19.2 An appropriate incision and drainage of infection should allow dependent drainage as the patient lies recumbent in bed

development of healthy granulation tissue. The goal of this initial surgical debridement is to convert an acute infection into a chronic wound. While negative cultures following initial debridement are preferred, it is not a prerequisite for definitive surgery and wound closure as additional surgical debridement is performed at the time of wound closure.

Forefoot Procedures

First Ray

Ulcerations of the first ray (hallux and first metatarsal) are among the most common sites for ulcerations. The primary reasons for this are the increased weight-bearing forces across this joint and abnormal biomechanics [22–24]. Excessive pronation leads to medial transfer of weight-bearing forces through the medial longitudinal arch, the first metatarsal, and ultimately the hallux [25]. Common sites of ulcerations include: (1) plantarmedial aspect of the hallux, (2) distal tip of the hallux, (3) directly plantar to the interphalangeal joint (IPJ) of the hallux, (4) directly plantar to the metatarsophalangeal joint (MTPJ), (5) directly plantar to the first metatarsal head, and (6) medial aspect of the first metatarsal head. Any structural deformity such as osteoarthritis, hallux limitus/rigidus, or severe plantar flexion will further alter the biomechanics of the joint increasing the susceptibility of this joint to ulceration. Assessing the underlying structural or mechanical cause of the ulceration is vital to understanding the reasons for ulceration and for selecting the most appropriate procedure.

Ulcerations of the hallux, either plantarmedial or directly plantar to the interphalangeal joint, are commonly related to abnormalities in the first MTPJ, either structural or mechanical. This is often manifested clinically by the presence of callus on the medial aspect of the hallux ("medial pinch" callus) or limitation of motion at the first (MTPJ) (i.e., hallux limitus/rigidus) (Fig. 19.3). The IPJ will hyperextend to compensate for this lack of motion [26, 27]. Other less common causes for ulceration are an enlarged medial condyle on the distal phalanx or the presence of an interphalangeal sesamoid bone, in which case the ulceration is typically directly plantar to the interphalangeal joint.

The choice of surgical procedure depends on the underlying cause. When the cause of the ulceration is related to lack of adequate motion at the MTPJ, motion can be restored by way of an arthroplasty of the hallux interphalangeal joint (HIPJ) or of the first metatarsophalangeal joint (MTPJ) [28–30]. Resection of the head of the proximal phalanx relieves excessive plantar pressure, increases motion, and allows for resolution of the ulceration. This procedure can also be employed when osteomyelitis of the head of the proximal phalanx is suspected. In cases where there are significant degenerative changes at the level of the first MTPJ or complete lack of dorsiflexion, it may be best to resect the base of the proximal phalanx to restore motion at this joint.

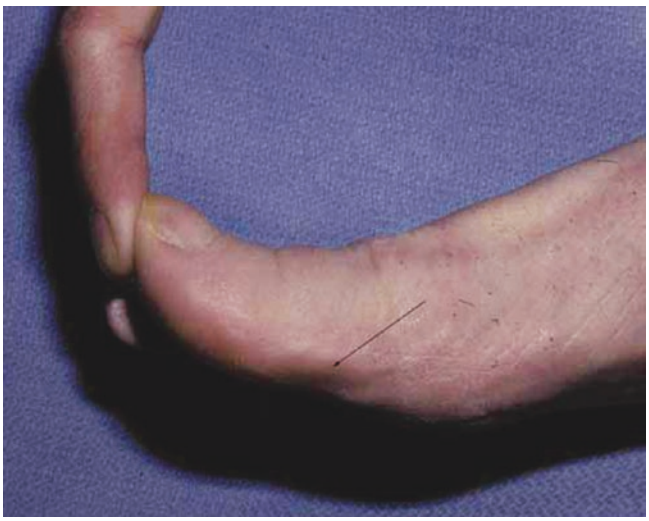

Fig. 19.3 A common location for ulcerations of the great toe is on the plantarmedial aspect of the interphalangeal joint of the hallux. The most common reason for these ulcerations is a hallux limitus/rigidus

Ulcerations directly plantar to the first metatarsal head are common. One possible approach to resolving these ulcerations is by excising one or both sesamoid bones [31]. During the propulsive phase of gait, the sesamoids will migrate distally to be under the first metatarsal head, thus becoming more prominent. In patients with motor neuropathy and an intrinsic minus foot, the sesamoids could serve as a potential pressure point and site of ulceration. A tibial and/or fibular sesamoidectomy is indicated for a chronically recurrent ulceration that is directly plantar to the first metatarsal head. Additionally, there should be no clinical or radiographic signs of osteomyelitis of the first metatarsal head. If osteomyelitis of the first metatarsal head is suspected, this is best treated with resection of the first MTPJ. Additionally, the presence of significant degenerative changes of the first MTPJ is best treated with an arthroplasty of the first MTPJ (Keller procedure) [32]. A rigid plantarflexed first ray is a relative contradiction to a sesamoidectomy and may require an adjunctive procedure (e.g., dorsiflexing first metatarsal osteotomy). It is critical to differentiate grade 2 ulcerations from grade 3 ulcerations with involvement of the first MTPJ. Ulcerations that probe directly into the joint or to bone with a blunt stainless steel probe can be considered to be clinical evidence of osteomyelitis (Fig. 19.4) [33–35]. In this case the procedure of choice should be one that completely resects all infected bone. The joint resection can be performed through a dorsal approach, leaving the plantar ulcer to heal by secondary intention. Alternately, the first MTPJ may be resected through a plantar approach by excising the ulcer followed

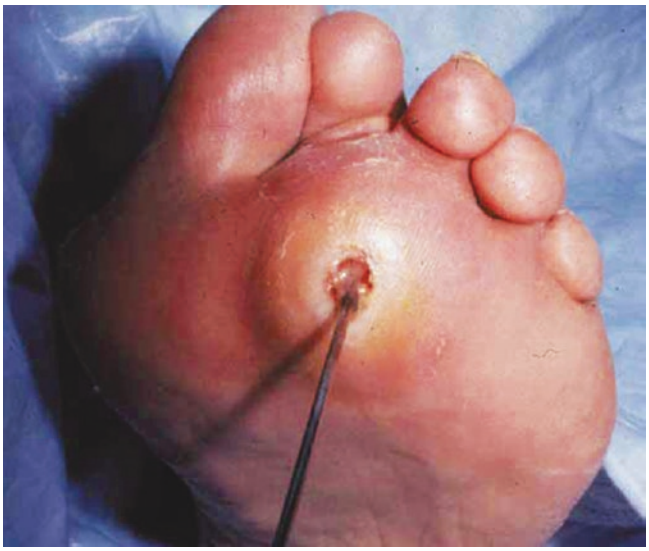

Fig. 19.4 The presence of synovial drainage from an ulceration is indicative of joint involvement and requires resection of that joint

by primary closure with full thickness, nonabsorbable suture. There are clear advantages to utilizing this approach. By excising the ulceration, all infected, nonviable tissue is removed. It also allows for excellent exposure of all potentially infected tissues, including the flexor hallucis longus tendon and the sesamoid bones which are commonly involved. Additionally, wounds that are closed primarily heal more predictably and with less scarring. As a rule, these wounds heal in 3–4 weeks. The healing rate of wounds which are allowed to heal by secondary intention cannot be predicted and are often dependant on size and depth. The longer these wounds remain open, the greater the risk of secondary infection as patient compliance diminishes the longer the ulcer remains open. While disadvantages exist to closing these wounds primarily as well, it is our philosophy that the benefits of primary closure outweigh the risks.

Approaching the foot plantarly, an elliptical incision is made excising the ulceration in toto. The ratio of incision length to width should be at least 3:1 to allow for tension-free closure. This incision is full-thickness and is carried down to the first metatarsal joint (Fig. 19.5). All necrotic and infected tissue should be excised at this time. At this point the flexor hallucis longus tendon will be visible. Typically focal necrosis within the body of the tendon is visualized, indicating infectious involvement. It is therefore best to sacrifice the tendon in order to prevent recurrence of the infection. Removal of the long flexor tendon will often require an adjunctive procedure of lengthening of the extensor halluces longus tendon on the dorsum of the foot. Failure to perform this could result in an extensus deformity of the great toe, making shoe fit difficult.

Once the tendon is removed, the sesamoids are visualized. They should be sacrificed as they are intra-articular

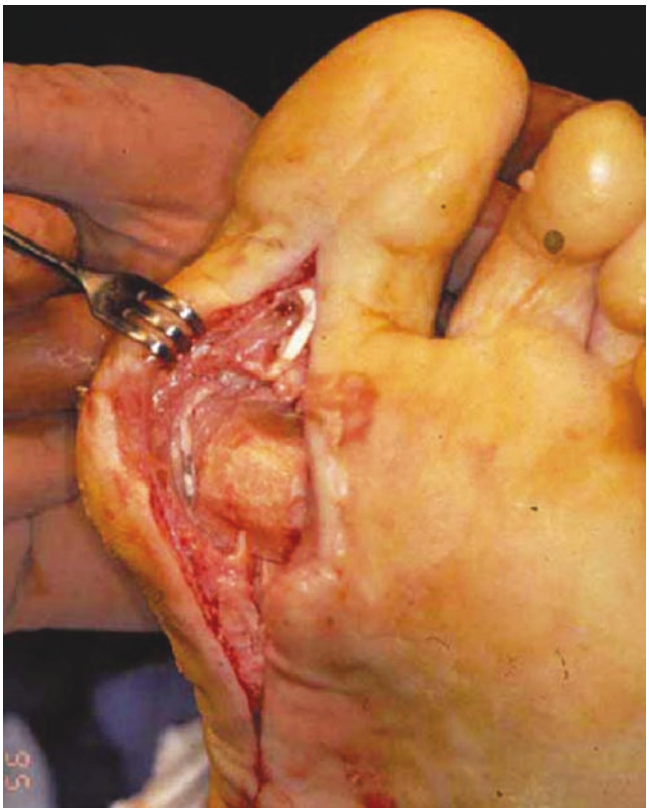

Fig. 19.5 Osteomyelitis of the first metatarsophalangeal joint is best addressed by elliptical excision of the ulcer with resection of the joint. Adequate resection of the first metatarsal should be performed to assure complete eradication of infected bone

structures and are in direct communication with the first MTPJ. The base of the proximal phalanx and the cartilage of the first metatarsal head are now resected. While it is preferred to maintain as much metatarsal length as possible, to maintain some weight-bearing function, the goal should be to resect enough metatarsal to remove all focus of osteomyelitis.

Closure is achieved by using full thickness nonabsorbable sutures. Nonabsorbable, monofilament 2–0 and 3–0 suture such as polypropylene (Prolene®) is preferred. Sutures are evenly spaced and used to coapt skin edges with as little tension as possible. Deep sutures are avoided as they can serve as a potential focus of infection and may be difficult to retrieve at a later date if necessary. One may consider packing the proximal 1.0 cm of the wound with a 2 × 2 gauze sponge to allow for drainage and avoid the development of a hematoma. This is usually removed after 24–48 h and allow the wound to heal by secondary intention. The postoperative care mandates a period of total non-weight-bearing of at least 4 weeks. Early ambulation will result in wound dehiscence, persistent drainage, postoperative infection, and possible hypertrophic scar. The sutures are left in place this entire time.

Lesser Digits

Motor neuropathy in patients with diabetes can result in atrophy of the intrinsic muscles of the foot [36]. This can result in forefoot deformities such as hammertoes and clawtoes (Fig. 19.6). In the presence of sensory neuropathy, ulcerations can develop over the proximal interphalangeal joint, at the distal tip of a toe or on adjacent sides of toes. With the exception of the second toe, amputation of a lesser toe rarely results in long-term complications. Loss of the second toe can lead to a hallux valgus deformity. But when an ulceration is discovered early and treated aggressively, amputation of the toe can be avoided, thus maintaining function as well as appearance.

Hammertoes are either classified as reducible or nonreducible. A reducible hammertoe implies the deformity is being held by contractures of the soft tissues while a nonreducible deformity suggests there has been bone and joint adaptation as well as extensive soft tissue contractures. Reducible deformities are often amenable to correction by a tenotomy of the corresponding flexor tendon. This can be performed in the office by making a small stab incision just proximal to the flexor crease of the affected toe with a #6100 Beaver blade. The blade is advanced until the flexor tendon can be palpated. The blade is then used to transect the flexor tendon in a transverse direction while applying a gentle dorsiflexing force on the toe. This puts the flexor tendon under tension making it easier to palpate. Once the tendon is released, the digit will relax and straighten. The toe is then splinted for approximately 1 week to maintain the correction.

Because of bone and joint adaptations, nonreducible deformities require a more aggressive approach. Resection of the phalangeal head along with release of soft tissue contractures are necessary to fully reduce the deformity. This may be combined with excision of an ulceration if present.

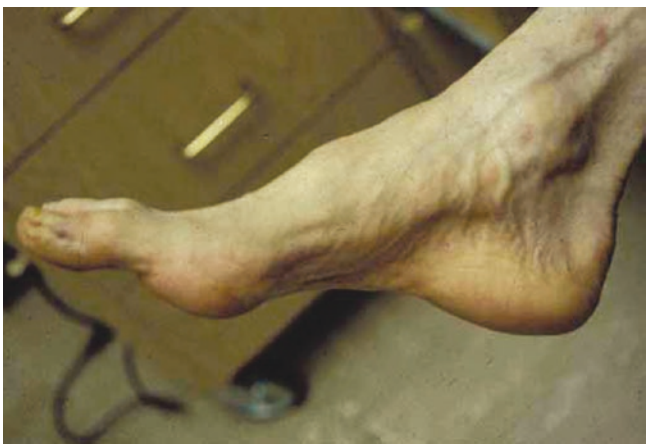

Fig. 19.6 Motor neuropathy is characterized by wasting of the intrinsic musculature in the arch of the foot. This typically results in deformities such as hammertoes, clawtoes, or plantarflexed metatarsals

In long-standing hammertoe deformities there may be a concomitant contracture at the level of the metatarsophalangeal joint (MTPJ), often with subluxation or dislocation at this level. When dislocated, an area of high pressure can develop on the ball of the foot under the corresponding metatarsal head. This manifests with callus or even ulceration. Failure to recognize this fact can lead to incomplete correction of the deformity and failure to resolve the ulceration. Correction of this deformity proceeds in a stepwise approach. First, a tenotomy and capsulotomy of the joint is performed. If the joint cannot be relocated following soft tissue release alone, a shortening osteotomy of the metatarsal should be considered to relocate the joint and relieve the plantar pressure.

Lesser Metatarsal Procedures

The area under the lesser metatarsal heads is the next most common location for diabetic foot ulcerations. Common causes for high foot pressures and ulcerations in this location include abnormal foot mechanics, plantarflexed metatarsals, limited joint mobility, and prior surgical intervention [37–39]. While definitive studies on ulcer incidence and location do not exist, it appears that the second metatarsal is more susceptible to ulceration than the other lesser metatarsals. This is most likely due to the second metatarsal's dependence on the mechanics of the first ray. When excessive pronation of the medial column occurs, there is increased weight transfer and pressure to the lateral metatarsals [25]. This is manifested by the development of callus under the second metatarsal head. After the second metatarsal, the typical order of ulcer development is the third metatarsal then the fifth followed by the fourth.

Selection of surgical procedures for ulcerations under the metatarsal heads requires careful evaluation of the ulcer. As with the first metatarsal, a critical determinant in the surgical management of these ulcerations is the presence or absence of osteomyelitis.

Lesser Metatarsal Osteotomy

A lesser metatarsal osteotomy can serve as a valuable adjunct in the management and resolution of these ulcerations [40]. The primary goal of these procedures is to alleviate areas of high focal pressure. The presence of a chronically recurrent ulceration under a metatarsal head without direct extension into bone is the primary indication. The metatarsal is approached through a dorsal incision. Dissection is carried down to the surgical neck of the metatarsal. Once identified, a through and through osteotomy is made at this level. A variety of techniques have been

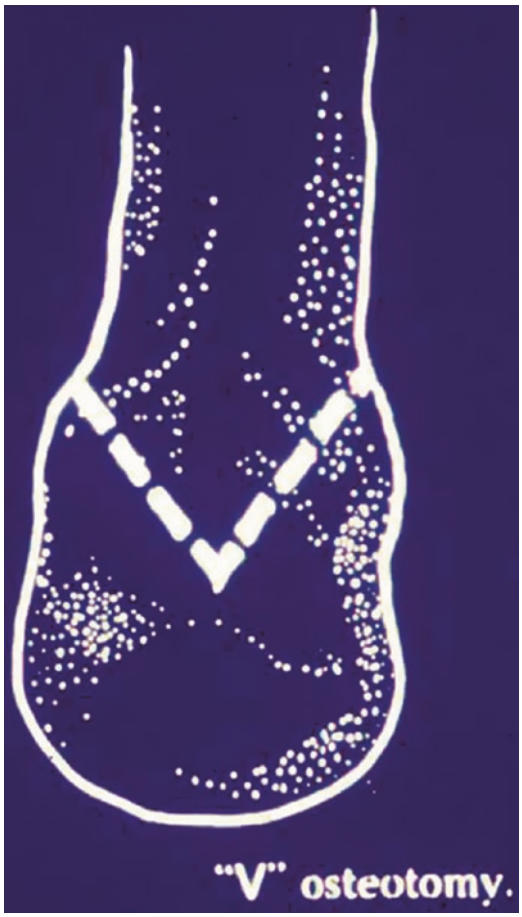

Fig. 19.7 A dorsal to plantar V-osteotomy through the surgical neck of the lesser metatarsal allows for adequate relief of plantar pressure overlying an ulceration. The medial and lateral wings of the "V" decrease the risk of medial or lateral dislocation of the metatarsal head

described for this osteotomy. Our preferred techniques are either the V-type osteotomy with the apex directed toward the joint or the Weil osteotomy with screw fixation (Fig. 19.7). The dorsal to plantar V-osteotomy provides a stable bone cut resistant to medial or lateral dislocation. A small collar of bone can be resected allowing for both shortening and elevation of the metatarsal if necessary. This is often desired when the metatarsophalangeal joint is either subluxed or dislocated. The metatarsal head is then elevated to the same level of the adjacent metatarsals. Fixation of the osteotomy with a 0.045 Kirschner wire is recommended. However, in the presence of an open ulceration, the use of internal fixation should be used cautiously as this may increase the risk for deep infection. Fixation and stability can alternately be achieved by impacting the head onto the shaft. The patient is kept non-weight-bearing for 4–6 weeks to allow for primary bone healing.

The Weil osteotomy can also be performed in this clinical situation [41]. In this approach, a dorsal-distal to plantar-proximal osteotomy at a 45° angle is made at the level of the surgical neck. It can be fixated with a single 2.0 cortical screw (Fig. 19.8). The advantage of the Weil osteotomy is that it can shorten the metatarsal with little risk of dorsal dislocation. The Weil osteotomy works well in patients with a relatively normal to flatfoot. However, in patients with a rigid anterior cavus foot, the amount of proximal translocation may not be enough to resolve the ulceration. In those patients, the V-osteotomy is the preferred procedure.

Complications following metatarsal osteotomies include transfer calluses or ulcerations and stress fractures of adjacent metatarsals. These most commonly result when the metatarsal head is elevated above the plane of the adjacent metatarsals. The risk of transfer problems can be reduced if the patient is fitted with an accommodative custom orthosis postoperatively. This will allow for more even distribution of weight-bearing forces across all metatarsal heads. Shoe gear modification may also assist in this role.

Lesser Metatarsal Head Resection with Ulcer Excision

An alternate approach for relieving plantar pressure is to resect the offending metatarsal head entirely. While this will result in resolution of the ulceration, this carries a high incidence of transfer lesion or ulceration. For this reason, it is preferred to perform this procedure only when osteomyelitis of the metatarsal head is suspected and there is no alternative but to resect the offending metatarsal head.

Resection of the metatarsal head can be approached through a dorsal linear incision centered directly over the metatarsal head. It should be remembered that the base of the corresponding proximal phalanx should also be resected as this structure is contiguous with the metatarsal head and is most likely involved as well. The ulcer is then allowed to heal by secondary intention.

Alternately, the metatarsal head may be resected through a plantar approach while excising the ulceration at the same time. The advantage of this approach is that all necrotic and infected tissue is excised and all tissue can be directly inspected (Fig. 19.9). Following resection of the metatarsal head, the wound is closed primarily as previously described for first MTPJ resection.

Postoperatively, sutures are left in place for a minimum of 3 weeks and the patient is maintained totally non-weight-bearing for 3–4 weeks. Antibiotics are continued until the sutures are removed. Long-term complications include possible transfer lesions or ulcerations and stress fractures due to the altered weight-bearing surface. It is therefore recommended that patients be fitted with an appropriate orthotic device to distribute pressures evenly.

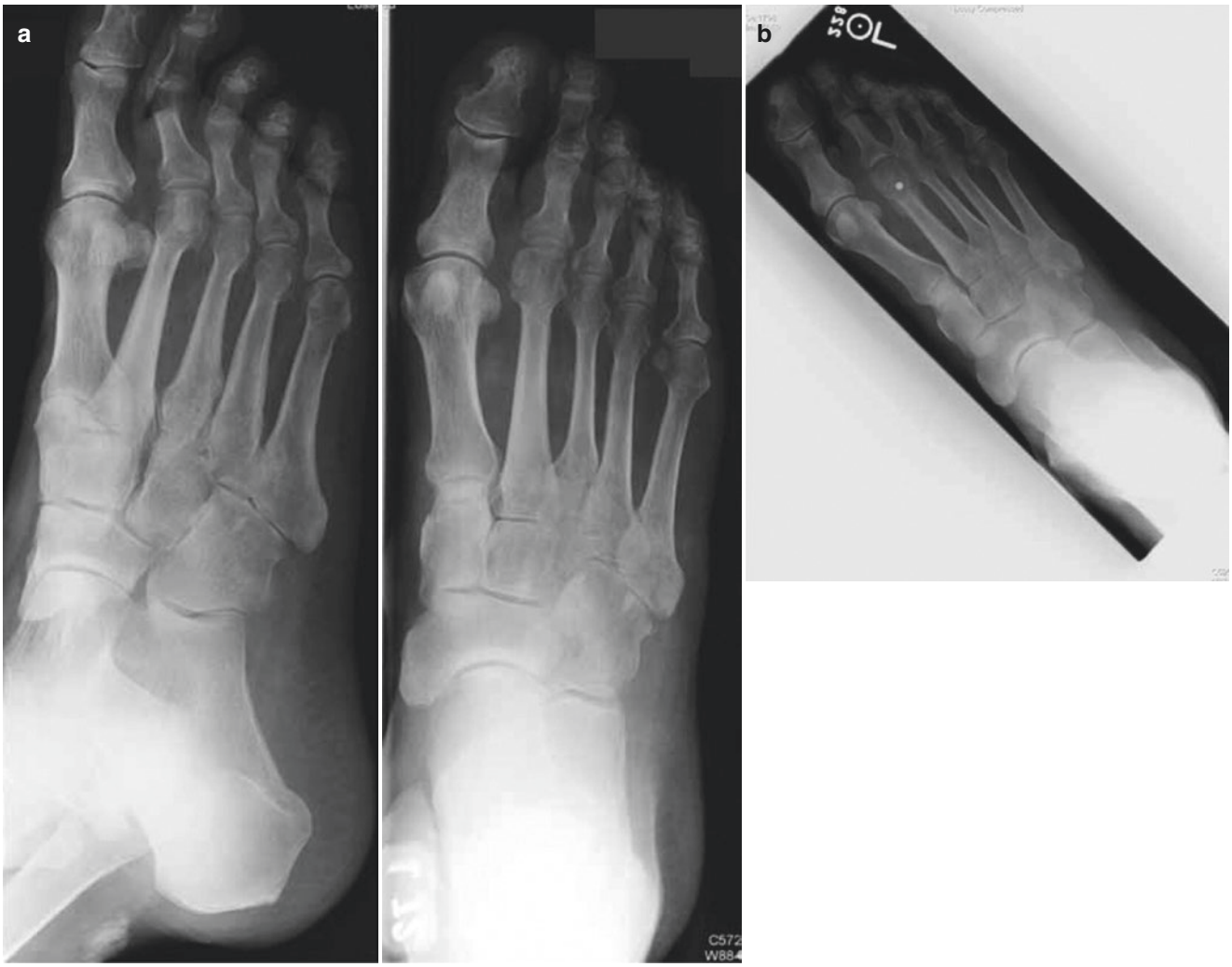

Fig. 19.8 An alternative osteotomy is the Weil where the bone cut is directed at a 45° angle from dorsal distal to plantar proximal direction and is fixated with a single 2.0 screw . (**a**) is preoperative x-ray showing long 2nd metatarsal; (**b**) shows deformity corrected with osteotomy and placement of 2.0 screw for fixation

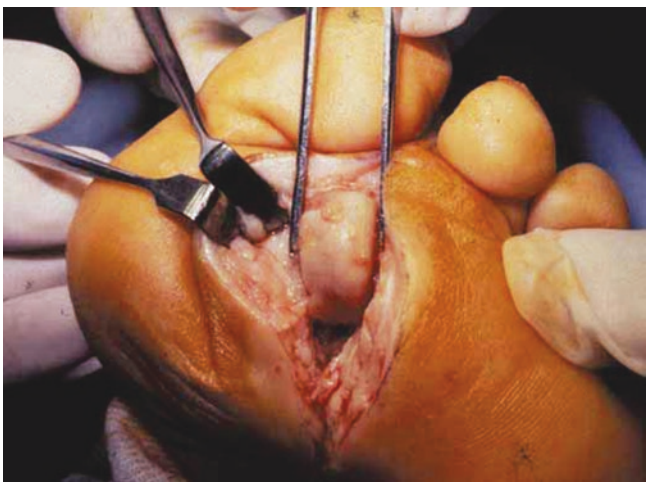

Fig. 19.9 An osteomyelitic lesser metatarsal head can be resected through a plantar elliptical incision excising the ulceration in toto

Panmetatarsal Head Resection

Weight-bearing forces are designed to be evenly dispersed across all metatarsal heads. This interdependence between the metatarsal heads has been previously described first by Morton and later by Cavanagh [22, 23]. Disruption of this relationship will alter normal weight distribution and consequently peak pressures. Various factors can affect the weight distribution across the metatarsals such as fractures resulting in dorsiflexed or shortened metatarsals, the atrophic form of Charcot neuroarthropathy resulting in dissolution of metatarsal heads or prior surgical resection of one or more metatarsal heads for osteomyelitis.

The recidivistic nature of diabetic foot disease makes multiple metatarsal procedures common in this patient population. Osteomyelitis of the forefoot was previously treated by transmetatarsal amputation. This procedure was popularized

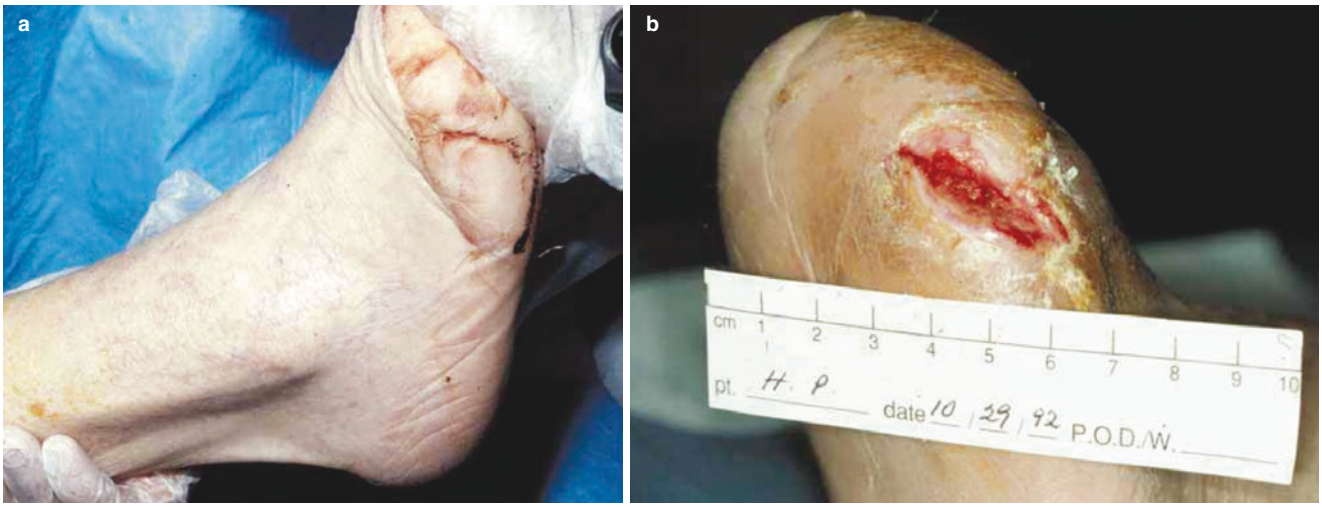

Fig. 19.10 (**a**) A common complication following transmetatarsal amputation is contracture of the Achilles tendon and subsequent equinus deformity. This can lead to characteristic lesions at the distal end of the TMA. (**b**) A distal lateral ulceration of a TMA with an underlying equinovarus deformity

by Dr. Leland McKittrick of the New England Deaconess Hospital and was responsible for saving thousands of limbs [42]. It is not without its complications however. Ulcerations at the distal stump and equinovarus contractures are common long-term complications (Fig. 19.10a, b). Additionally, patients have difficulty psychologically accepting this procedure at times because it will often require special shoegear that draws attention to the fact they have had an amputation.

The panmetatarsal head resection (PMHR) and its variations were originally described for the treatment of painful lesions in patients with arthritis [43–46]. Jacobs first described the use of the panmetatarsal head resection in patients with diabetes for the successful treatment of chronic neuropathic ulcerations [47]. This report was subsequently followed by a report by Giurini et al. where a larger series of patients were studied with similar results. Additionally, an alternate technique was described [48]. Over the years, the panmetatarsal head resection has replaced the TMA as the procedure of choice in patients with recurrent ulcerations following prior surgical resection of metatarsal heads [49, 50].

The panmetatarsal head resection is rarely a procedure of first choice. The primary indication is the presence of chronically recurrent neuropathic ulcerations on the plantar aspect of the foot following prior metatarsal head resections or ray amputations. It is our belief that if two or more metatarsals have already been resected or need to be resected to eliminate osteomyelitis, the patient would be best served by a panmetatarsal head resection (Fig. 19.11). At first this may appear to be a drastic, aggressive approach. However, experience has shown that this approach may actually spare patients additional trips to the operating room for transfer ulcerations.

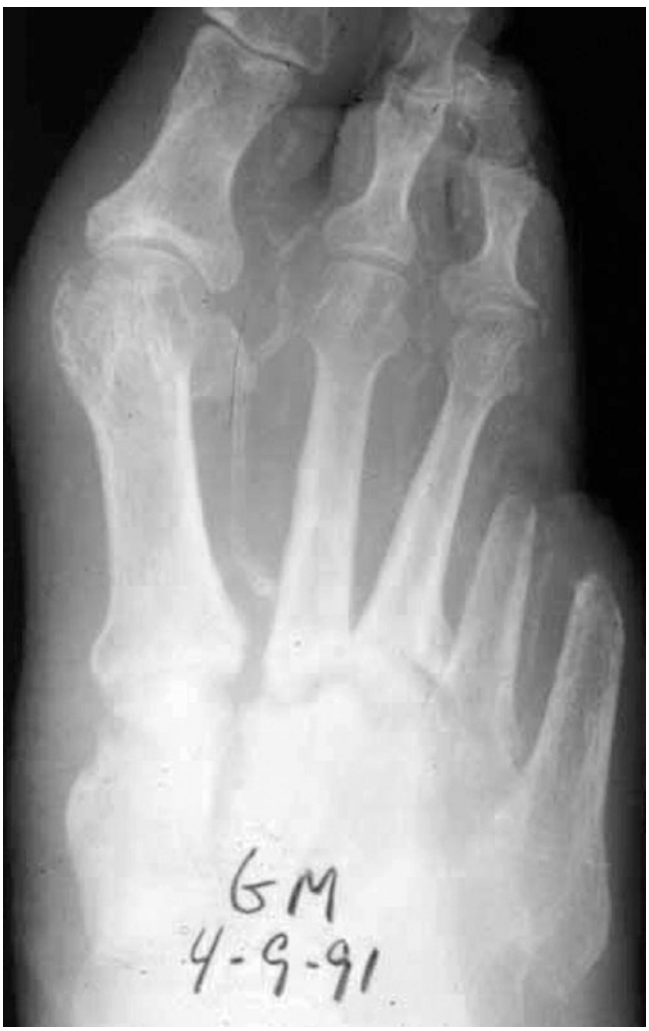

Fig. 19.11 Prior resection of two metatarsal heads and the presence of osteomyelitis of a remaining metatarsal head is indication for panmetatarsal head resection

Various surgical approaches have been described for the panmetatarsal head resection. Dorsal approaches, plantar approaches or a combination of the two have been performed with equal success [50]. Our preferred approach is the four incision dorsal approach: one incision directly over the first metatarsal, one between the second and third metatarsals, one directly over the fourth metatarsal, and one directly over the fifth metatarsal. This approach allows for adequate exposure of all metatarsal heads, decreases the potential for retraction injury on the skin edges, and maintains adequate skin islands so as not to affect vascular supply. The most common approach is to combine a dorsal incision with a plantar incision since the primary indication is the presence of an open ulceration with osteomyelitis. The plantar wound and all necrotic tissue can then be excised, the involved metatarsal head(s) can be resected, and the wound closed primarily as previously described.

The surgical technique for resection of the metatarsal heads has already been described. The most important technical point to remember in performing this procedure is to maintain the metatarsal parabola. This typically means that the first and second metatarsals are left approximately the same length while the third, fourth, and fifth metatarsals are each sequentially shorter. Failure to maintain this relationship can lead to recurrent ulceration. This may be difficult to achieve if there has been a prior metatarsal head resection. In that case the metatarsal parabola should be recreated with the remaining metatarsals. The extensor tendons dorsally or the flexor tendons plantarly are identified and are retracted. This maintains the function of these tendons during the gait cycle affording this procedure the prime advantage over the TMA.

Midfoot Procedures

Surgery in the region of the midfoot is most commonly necessary following foot deformities resulting from neuroarthropathic (Charcot) joint disease. The most common location of Charcot joint involves the tarsometatarsal (Lisfranc's) joints but other joints in the midfoot may also be affected [51, 52]. Instability at Lisfranc's joint often results in a rockerbottom deformity at the level of the midfoot, resulting in a plantarmedial ulceration. This is due to subluxation of the first metatarsal and medial cuneiform creating a plantar prominence. Ulcerations on the plantar and lateral aspect of the foot are not uncommon. These result from plantar extrusion of the cuboid from a Charcot process at the calcaneocuboid joint [51]. These pose a significant management problem as they are typically recalcitrant to conservative measures. There is no single surgical procedure that can be applied to all ulcers in this location. Therefore, a flexible approach to these lesions is required. Surgical approaches may involve simple ostectomy with or without fasciocutaneous flap or primary arthrodesis of unstable joints [53].

Ostectomy

This is the simplest approach to chronic plantar ulcerations of the midfoot. This is reserved for those deformities that have their apex directly plantar to the first metatarsal-medial cuneiform joint and where the midfoot is not hypermobile. The depth of the ulceration dictates the best surgical approach. A direct medial incision centered over the joint is preferred when the ulceration is superficial and does not involve bone. This allows for excellent visualization of the joint and the prominent bone. The prominence is resected from medial to lateral either with an osteotome or with a saw. The goal should be to remove an adequate amount of bone to alleviate the plantar pressure and not create a new bony prominence which could create a new source of irritation and ulceration, thus negating the benefits of this procedure.

Ulcerations which communicate with bone and show signs of osteomyelitis clinically are best managed by excision of the ulceration with bone resection and primary closure of the ulceration. In addition to removing the infected bone, the ability to close the ulceration primarily without tension is an additional goal. This approach is best employed when the ulcer is located either plantar central or plantar lateral in the midfoot. The most likely etiology for these ulcerations is plantar displacement of the cuboid. When the ulceration measures less than 2.5 cm, this direct surgical approach can be used. The use of closed suction irrigation is also recommended in order to prevent hematoma formation which can lead to wound dehiscence or infection.

One of the more difficult ulcerations to manage is an ulcer located centrally in the midfoot secondary to plantar subluxation of the cuboid bone. This is the type 5 of the Harris and Brand classification of Charcot joint disruption (pattern II in the Sanders classification) and has been described as being very resistant to conservative care [51]. Resolution of these ulcerations often require surgical intervention of some type.

Exostectomy with Fasciocutaneous Flap

Ulcerations greater than 2.5 cm in diameter are difficult to close primarily in a tension-free manner. In these cases alternate techniques for wound closure should be sought. These ulcerations are typically excised circumferentially to the level of the cuboid bone. This allows removal of all necrotic, infected tissue as well as any hyperkeratotic margins bordering the ulcer. The joint capsule and periosteum of the cuboid are next encountered which are reflected off

the underlying bone, presumably the cuboid. This will expose the peroneal groove of the cuboid bone which is usually the culprit in these ulcerations. The peroneus longus runs through this groove. When possible this tendon should be retracted out of harm's way. On rare occasions, however, it may be necessary to sacrifice the peroneus longus in order to gain adequate exposure of the bony prominence. The peroneal groove is next resected with the use of an osteotome and mallet. Once completed, the wound should be carefully inspected for any remaining bony prominence or bone spicules which can serve as a new point of pressure and possible ulceration.

This procedure will often leave a relatively large dead space which can serve for the collection of a hematoma. It is best to fill this dead space with a muscle flap which will serve two purposes: (1) it will decrease the dead space following the bony resection and (2) it will provide a layer of soft tissue between the underlying bone and the overlying skin (Fig. 19.12). The flexor digitorum brevis muscle is well suited for this purpose because of its anatomic proximity to the resected bone and ease of dissection. The muscle is rotated laterally to cover the cuboid. A full thickness fasciocutaneous flap based on the medial plantar artery is rotated from medial to lateral to cover the actual ulcer site. A split thickness skin graft is then used to cover the donor site in the medial arch (Fig. 19.13).

Six weeks of total non-weight-bearing is required for adequate healing and incorporation of the flap. This is followed by an additional 2–4 weeks of protected weight-bearing in a surgical shoe with a molded orthotic device. Long-term care requires the use of plastizote orthoses and modified shoegear.

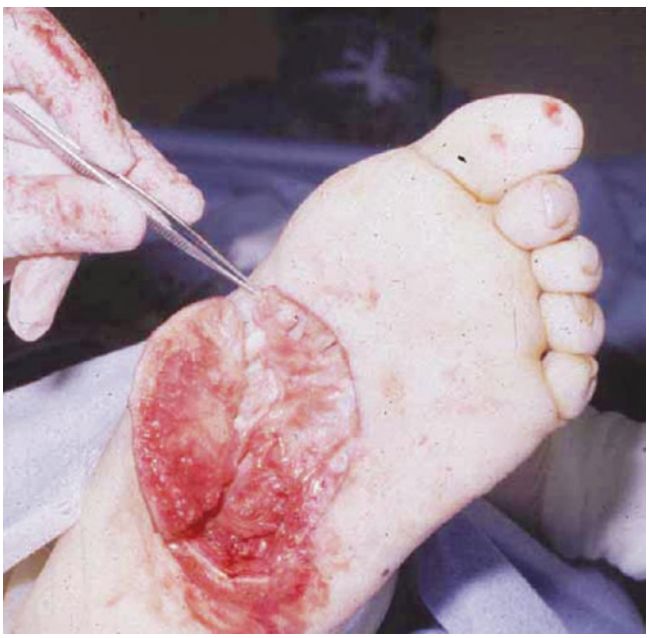

Fig. 19.12 The flexor digitorum brevis muscle is commonly used for closure in large plantar ulcerations following ulcer excision and exostectomy of the offending bone

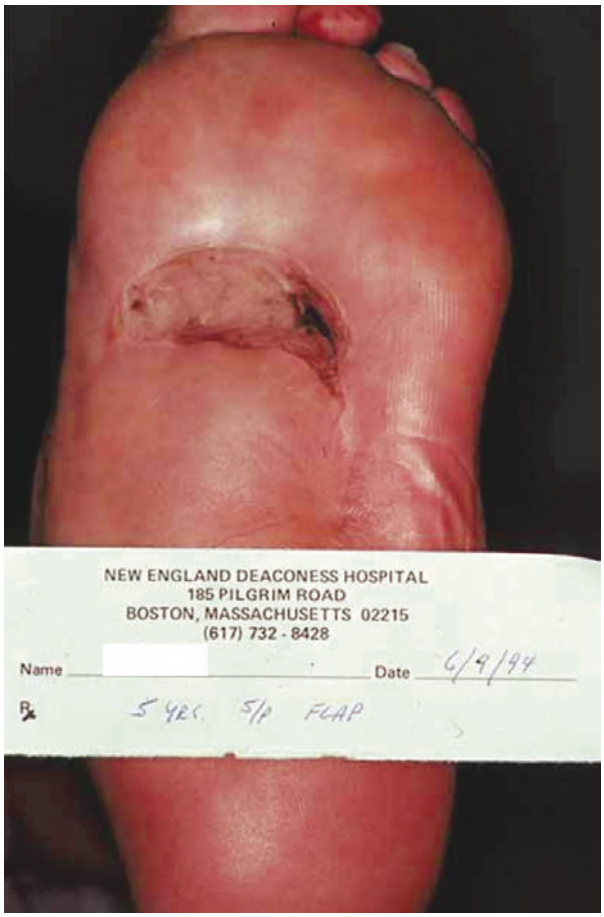

Fig. 19.13 Patient who is 5 years status post cuboid exostectomy with an interpositional muscle flap and a rotational fasciocutaneous flap

These types of flaps in the foot have become relatively infrequent since the introduction of negative pressure wound therapy (NPWT), also referred to as vacuum assisted closure (VAC). Negative pressure wound therapy was first introduced in the United States in 1997 [54–56]. Since its introduction, it has been extensively used in large circumference wounds with significant depth in order to promote granulation, decrease the number of dressing changes, and avoid more extensive procedures. As a result, there has been a significant reduction in the number of rotational flaps or free tissue transfers needing to be performed [57].

Lisfranc's Joint Arthrodesis

When the Charcot process results in significant bone loss and instability at the first metatarsal-medial cuneiform, exostectomy may result in further instability and continued collapse of this segment. In these circumstances

stabilization of the joint in the form of primary fusion may be a better alternative.

The joint is best approached through a direct medial incision. This allows adequate exposure of the dorsum of the joint as well as the plantar surface. Any remaining articular cartilage is resected with a sagittal saw. Often times the bone cut on the first metatarsal side can be slightly angulated from dorsal-proximal to plantar-distal allowing plantar flexion of the first metatarsal, restoring the weight-bearing function of the first ray. In addition, any plantar bony prominence can also be resected from medial to lateral.

Fixation of the joint is best achieved with a medial plate and interfragmentary screw or crossed screws to provide rigid internal fixation and compression (Figs. 19.14 and 19.15a, b). Other forms of fixation may include crossed 0.062 Kirschner wires, bone staples, or intramedullary screw which will be described later in this chapter. It is advisable to insert a Jackson-Pratt drain to prevent the accumulation of a hematoma.

The postoperative course includes immobilization and non-weight-bearing. While there is no standard length of immobilization and non-weight-bearing, the patient can expect to be non-weight-bearing on average 3 months. Partial weight-bearing begins when serial X-rays show early trabeculation across the first metatarsal-medial cuneiform joint. Continued weight-bearing is allowed as long as both clinical

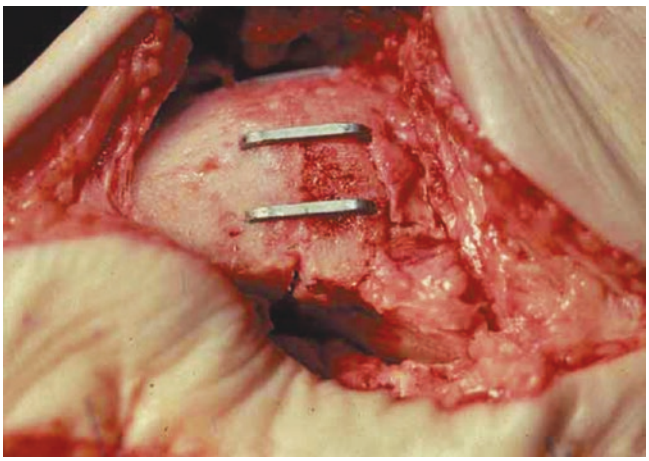

Fig. 19.15 Fusion of the first metatarsal-medial cuneiform joint for an unstable Charcot joint complicated by recurrent ulceration can be achieved by use of staples

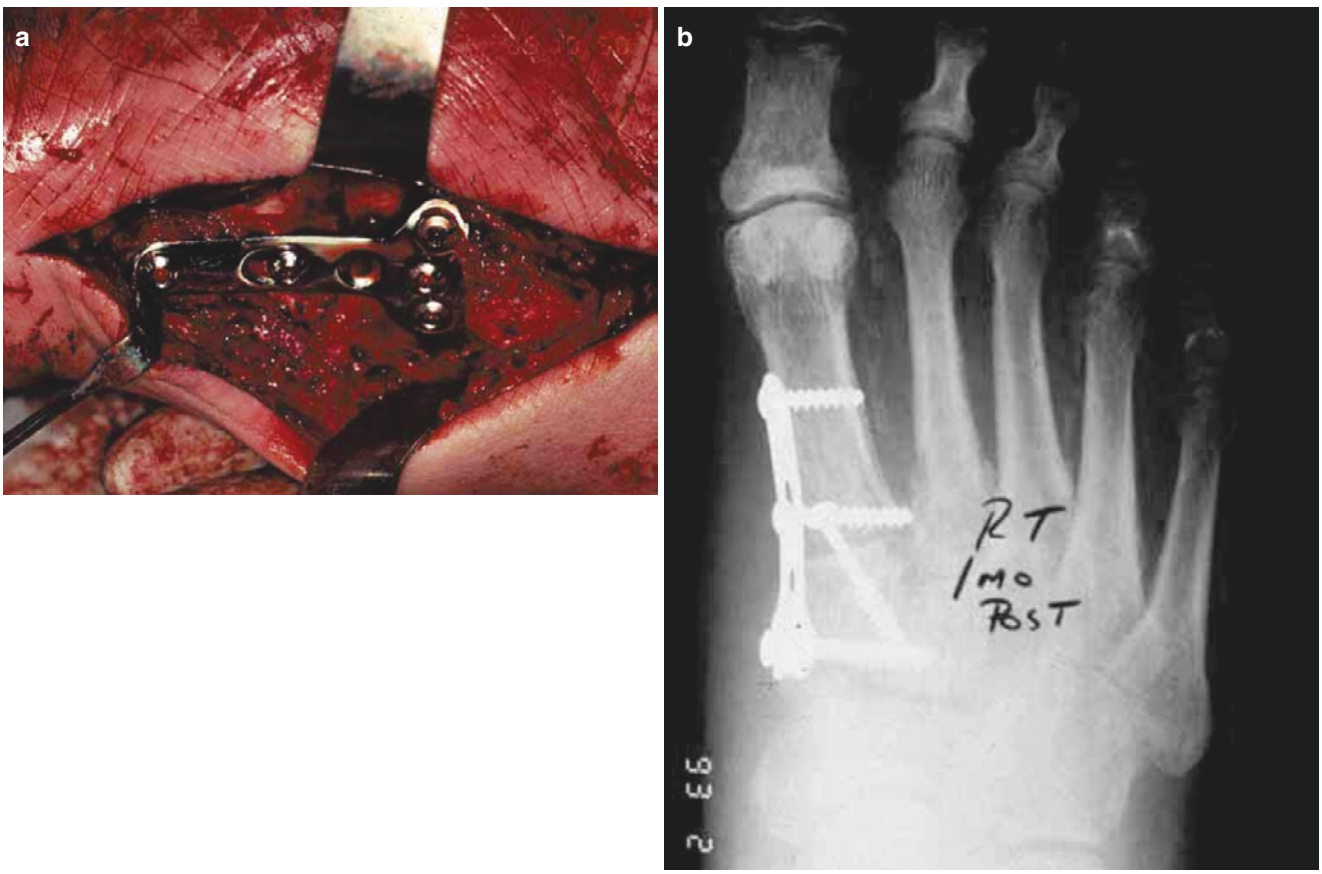

Fig. 19.14 (**a**) A T-plate with an interfragmentary screw is another acceptable form of fixation of the first metatarsal-medial cuneiform joint in the presence of unstable Charcot joint. (**b**) Radiograph of patient with T-plate and interfragmentary screw across the first metatarsal-medial cuneiform joint

and radiographic evaluation suggests continued healing of the fusion site.

Charcot joint disease will often affect the entire Lisfranc's joint complex, i.e., all five tarsometatarsal joints. In the case of severe midfoot instability stabilization of the entire midfoot is often necessary. The surgical approach to these deformities will be covered below under hindfoot procedures.

Calcanectomy

Heel ulcerations in patients with diabetes are not uncommon, are generally of moderate size, of long duration, and are associated with poor outcomes [58]. Due to the many comorbid conditions most diabetic patients display, periods of prolonged bed rest is not unusual. Without proper protection decubitus ulcerations can occur. However other causes for heel ulcers include blisters from shoe or cast irritation and heel fissures resulting from dry skin or puncture wounds. Regardless of the precipitating cause, the end result is prolonged disability and morbidity. In cases of bone involvement (i.e., osteomyelitis), below knee amputation can be the final outcome. Attempts to save this extremity and provide a limb capable of functional ambulation may involve excision of the ulceration and the calcaneus, either partial or subtotal.

The goals of the calcanectomy should include excision of all necrotic and infected soft tissue, resection of any and all infected bone, and primary closure of the wound whenever possible. Resection of large amounts of bone may be necessary in order to achieve primary closure. Hindrances to primary closure include the lack of mobility of the surrounding soft tissue and severe tissue loss from infection. In these cases, a more creative approach including rotational skin flaps, free tissue transfers, or NPWT may be needed.

The majority of times this procedure is performed for osteomyelitis. It is therefore critical that adequate bone is removed to eliminate the infection. It is also important that no plantar prominence be left behind which could serve as an irritant to the soft tissue and result in re-ulceration. In resecting the calcaneus, the Achilles tendon is often encountered. Depending on the extent of infection, it may need to be debrided or even released. While one may be tempted to reattach the tendon, it is rarely advisable to do so. Advancement of the Achilles tendon would require the introduction of foreign materials such as screws or anchors which could serve as a nidus of recurrent infection. In those cases where the Achilles tendon is detached, it will often fibrose to the surrounding tissues and provide some degree of plantar flexion (Fig. 19.16a, b).

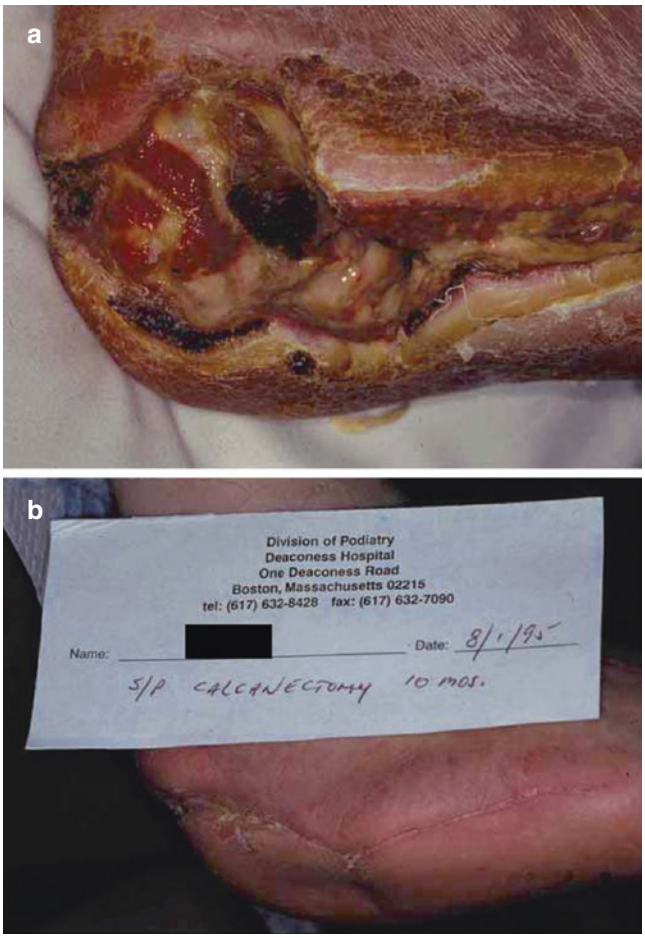

Fig. 19.16 (**a**) Osteomyelitis of the calcaneus with resultant soft tissue loss is a common cause of lower limb amputation. (**b**) Patient in 16**a** following partial calcanectomy with excision and debridement of infected, necrotic tissue and primary closure. Successful eradication of infected bone resulted in limb salvage

Hindfoot Procedures

Surgical procedures of the hindfoot are most commonly performed for reconstruction of unstable Charcot joint disease and can be truly classified as limb salvage procedures. These include midfoot arthrodesis, triple arthrodesis, and pantalar arthrodesis. While not considered reconstructive surgical procedures, we will also include tendo Achilles lengthening in this section as it is often a necessary adjunctive surgical procedure. Indications for these reconstructive procedures include chronic, nonhealing ulcerations with underlying hindfoot deformity or instability, severe instability of the hindfoot making ambulation difficult at best or chronic heel ulcerations with underlying osteomyelitis. While there is high risk associated with these procedures, standard conservative measures are often inadequate to provide a stable foot resistant to ulcerations. These procedures are often performed when the only alternative is a major limb amputation.

Midtarsal Joint Arthrodesis

As previously stated, the most common location for Charcot joint disease is the tarsometatarsal joints, i.e., Lisfranc's

joints. These are the joints formed by the metatarsal bases and the cuneiforms and cuboid bone. These joints are supported by several small ligaments that connect these bones to each other. While the inciting event for the development of Charcot joint disease remains unclear, in the majority of cases disruption of these ligaments with or without fractures is a common feature (Fig. 19.17a, b). Because of absence of pain, the patient continues to ambulate on this unstable foot resulting in further destruction, displacement, and instability. The end result is a foot that is grossly misshaped, unstable to walk on, and at risk for ulceration, infection, and amputation. While initial treatment should consist of non-weight-bearing, immobilization, and bracing, many feet are so unstable that bracing actually poses a risk to the patient. It is in these cases that surgical intervention should be contemplated.

We previously described medial column fusion for single joint involvement. However, in most cases, Charcot will affect multiple joints. Dorsal displacement of the midfoot or hindfoot is common and therefore a more aggressive approach is needed. Intramedullary rodding ("beaming") has become a common method of stabilizing these deformities (Fig. 19.18). Large screws are inserted through the intramedullary canals of the first and fourth (sometimes third) metatarsals. These screws cross the tarsometatarsal joints into the respective tarsal bones. In those cases where the talonavicular joint is also involved, a single long screw can be used to cross both the first metatarsal-medial cuneiform joint and the talonavicular joint as part of a triple arthrodesis. The screws are inserted following appropriate resection and realignment of the involved joints [59, 60].

This beaming technique has the advantage of providing adequate realignment and compression of the affected joints. This is a very stable construct. The other advantage is it avoids excessive dissection of the joints. With the use of cannulated screws and using intraoperative X-rays, these screws can be accurately placed through small stab incisions, avoiding large wounds and excessive stripping of the periosteum.

Triple Arthrodesis

The incidence of Charcot joint disease involving the tarsal joints—talonavicular, calcaneocuboid, or subtalar—ranges from 1.8% to 37% depending on the reports [61–63]. Clinically, these feet appear with a rockerbottom deformity from plantar subluxation of either the talonavicular joint or the calcaneocuboid joint (Fig. 19.19). This can then lead to chronic ulceration. When faced with a significant degree of instability from this destructive process, the approach should include surgical stabilization of the involved joint or joints. This often requires fusion of the talonavicular joint, calcaneocuboid joint, and subtalar joint, i.e., triple arthrodesis.

The goal of a triple arthrodesis is to stabilize the foot and to reduce the deformity, thereby reducing the risk of

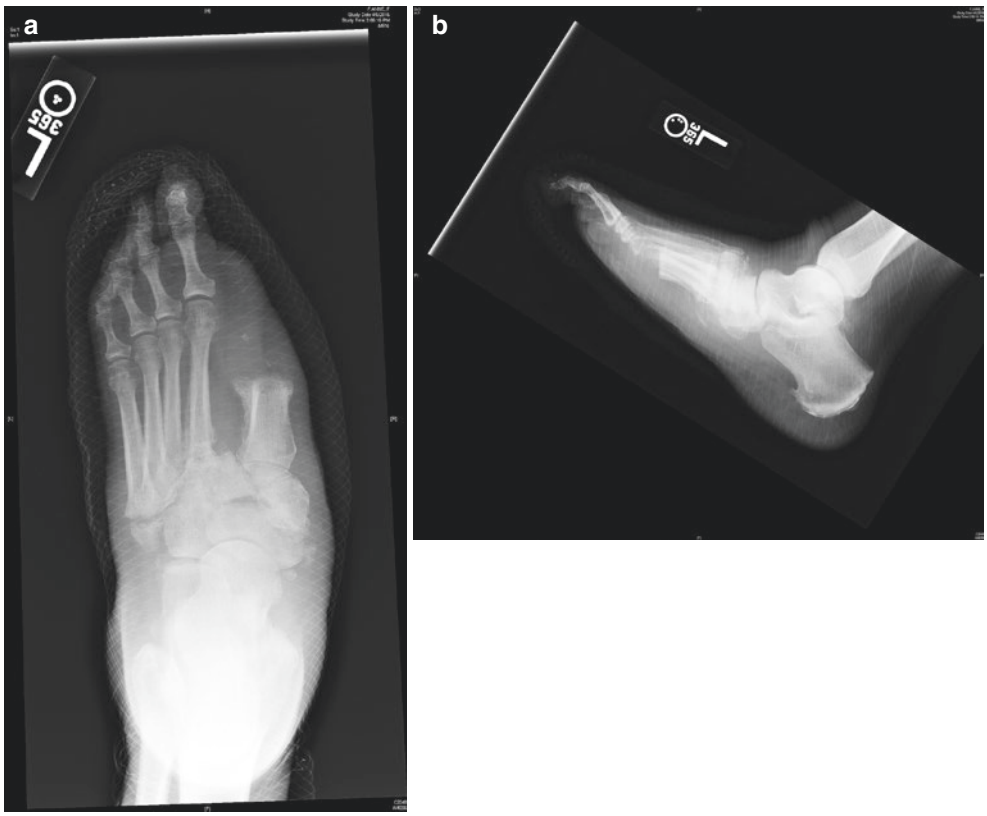

Fig. 19.17 (**a**) Disruption of tarsometatarsal ligaments resulting in lateral subluxation of Lisfranc's joint following first ray amputation. (**b**) Lateral view showing dorsal subluxation of Lisfranc's joint in addition to lateral subluxation

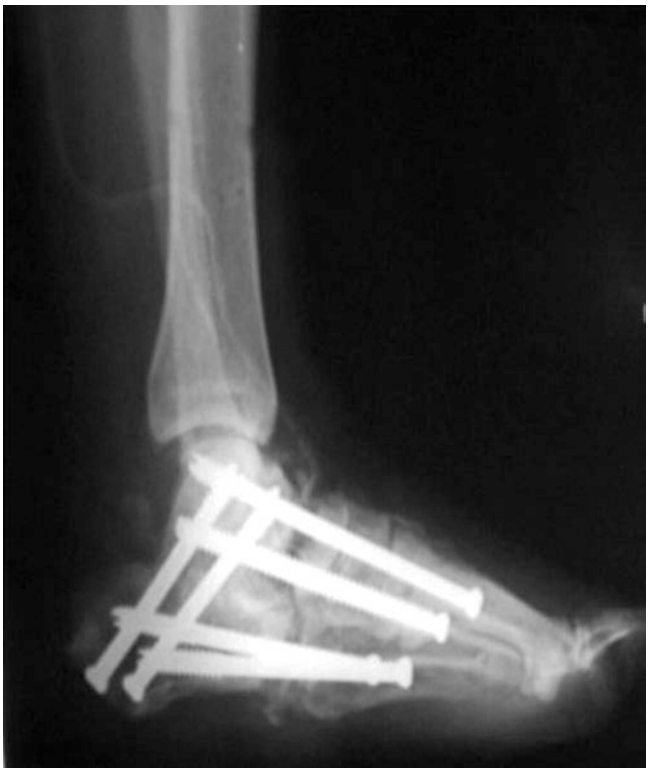

Fig. 19.18 The intramedullary rodding technique introduces large diameter screws through the metatarsals and across the hindfoot joints to achieve stability, primary fusion, and deformity

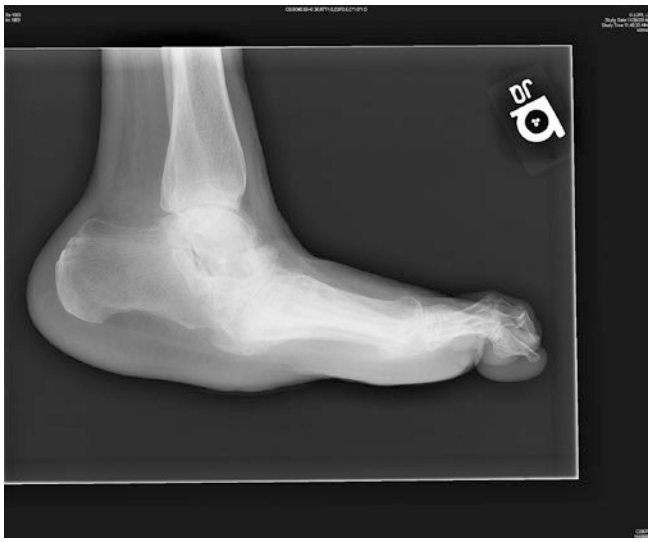

Fig. 19.19 Dislocation of the talonavicular joint from Charcot joint disease resulting in rockerbottom deformity and severe plantarmedial ulceration

recurrent ulceration. The surgery should be delayed until the acute phase has resolved and the Charcot joint has entered the coalescent phase. If an open ulceration is present, surgery should be delayed until all signs of acute infection are resolved.

The triple arthrodesis is performed in a standard fashion. The subtalar joint and the calcaneocuboid joint are approached through a lateral incision just inferior to the lateral malleolus and extending distally to the base of the fourth and fifth metatarsals. While it is possible to obtain adequate exposure of the talonavicular joint through this incision, a separate medial incision is often necessary to afford better exposure.

The cartilage is resected off all joint surfaces until bleeding bone is exposed. The joints are then apposed. If significant deformity exists, wedge resections through the joints may be required to adequately reduce the deformity. Additionally, significant bone resorption may have occurred as a result of the destructive process. In these cases bone graft may be necessary to fill the gaps between joint surfaces. This can be obtained from the iliac crest or from the bone bank.

The method of fixation is the surgeon's choice. Typically, the subtalar joint is fixated with a 6.5 mm cancellous screw. This screw can be introduced either from a dorsal approach through the neck of the talus or through a stab incision on the plantar surface of the heel. The screw is inserted over a guide wire from plantar to dorsal, across the subtalar joint into the body of the talus. While screws are preferred for the talonavicular and subtalar joints, staples or small plates can be used in the calcaneocuboid joint. Minimal to no gapping should be present. This should always be confirmed with an intraoperative X-ray to confirm the final position of all fixation devices, adequate joint apposition, and appropriate foot position. The position of the calcaneus should be neutral to slight valgus. The goal of surgery is correction of the deformity with good apposition of all joint surfaces and the creation of a plantigrade foot.

Postoperatively, the patient is placed in a posterior splint to immobilize the fusion site. This is replaced with a below-the-knee fiberglass cast usually 3–5 days of surgery. Total non-weight-bearing is maintained for a minimum of 3–4 months. Serial X-rays are obtained to evaluate bone healing and maintenance of postoperative correction and alignment. The patient is then advanced to gradual protected weight-bearing when X-rays show signs of bone union. Case reports suggest that the likelihood and rate of fusion may be improved with the use of electrical bone stimulation although prospective, randomized double-blinded trials are not available to determine overall efficacy [64].

Pantalar Arthrodesis

The ankle joint that has undergone severe destruction from Charcot joint disease is particularly problematic. This typically results in a flail ankle joint that makes ambulation extremely difficult if not impossible. This deformity may result from total collapse of the talar body, fractures through

the medial malleolus, lateral malleolus or both. Patients with these types of fractures will often be found ambulating directly on either the medial or lateral malleolus. This inherent instability will result in the development of chronic ulcerations and are extremely difficult to control with conservative care alone. The prognosis for these deformities is poor. In order for limb salvage to be achieved, primary fusion of the ankle and subtalar joints is necessary.

The surgical approach depends on the level and degree of destruction. If the primary level of instability and destruction involves the tibiotalar joint, isolated fusion of this joint may be sufficient. However most often, destruction of the other rearfoot joints is present. Therefore fusion of the ankle, talonavicular, subtalar, and calcaneocuboid joints (i.e., pantalar fusion) is necessary to provide a stable platform for ambulation. Once again, it is best to delay all surgical intervention until all signs of acute Charcot joint disease have resolved. Attempted fusion during the active, hyperemic phase of this disorder will not only make fusion technically difficult but may also result in failure to fuse.

A lateral incision which begins approximately at the midfibula and extends to the tip of the lateral malleolus offers adequate exposure of the ankle joint. If a pantalar fusion is to be performed, this incision can be extended distally to the calcaneocuboid joint. The fibula is typically osteotomized just proximal to the ankle joint line. The anterior aspect of the fibula is dissected free and reflected posteriorly. This preserves the vascular supply to the fibula. This will also allow the fibula to be used as a vascularized strut graft on the lateral side of the ankle joint. The ankle joint is now well visualized.

The articular cartilage is resected down to bleeding cancellous bone from the inferior surface of the tibia and the dome of the talus. The ankle joint is repeatedly manipulated so as to assess alignment of the foot. The joint surfaces are continually remodeled until optimal bone apposition and foot alignment is achieved. In cases where the talar body is deemed nonsalvageable, the tibia may be fused to the calcaneus or a bone graft can be inserted to fill the defect and accommodate for significant bone loss. Femoral head allograft may be used to fill this defect. However recent studies/reports have shown mixed long-term results using this technique [65–67]. If a pantalar fusion is being performed, the remaining hindfoot joints can be addressed at this time in the same manner as in a triple arthrodesis.

After all articular surfaces have been resected, the foot should be positioned so that all bone surfaces are in good apposition with minimal to no gapping. Care should also be taken to avoid any interposition of soft tissue. If the foot cannot be aligned properly or bone surfaces do not appose adequately, further remodeling of the bone should be performed. Once optimal alignment has been achieved, the ankle joint is ready for fixation. Internal fixation of the ankle joint can be performed in a variety of ways. This can be performed with the introduction of two 7.0 mm cannulated screws, from a plantar to dorsal direction through the body of the calcaneus and across the resected ankle joint. This will also fixate the posterior subtalar joint (Fig. 19.20). Ideally, the tips of the screw should purchase the cortex of the tibia. Other techniques may include crossed screws from the distal tibia into the talus and/or calcaneus, a blade plate on the lateral side of the calcaneus and across the anterolateral aspect of the tibia. Alternatively, a retrograde intramedullary nail may also be introduced across the ankle and subtalar joints from a plantar approach (Fig. 19.21) [66, 67]. When bone quality precludes the use of internal fixation, external devices for fixation are appropriate alternatives [68, 69]. The use of intraoperative imaging is critical in the placement of guide wires and for final fixation. It is critical that the calcaneus be positioned either in neutral or in slight valgus position. Any degree of varus should be avoided. After fixation of the ankle joint, the remaining rearfoot joints can be fixated as previously described.

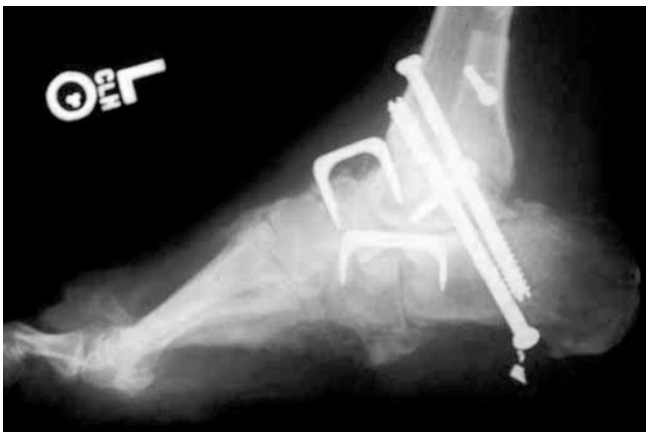

Fig. 19.20 Severe instability of the rearfoot due to Charcot joint often requires major reconstructive surgery of the hindfoot and ankle. A pantalar fusion was performed in this patient for severe cavoadductovarus deformity and chronic ulceration resulting from Charcot joint. Two 7.0 mm cannulated screws were used to fuse the subtalar and ankle joints

As with triple arthrodesis, the postoperative care is critical to successful limb salvage. Wound infection, dehiscence, and nonunion are the major complications seen with this procedure. Immobilization of the extremity immediately postoperatively can decrease the risks of these complications. Total non-weight-bearing in a fiberglass below-the-knee cast is required for a minimum of 4–6 months and should be changed frequently to prevent abrasions or cast irritations. Once it is felt fusion is sufficient to support weight-bearing, this should be instituted in a gradual protected manner. A return to protected weight-bearing will be dictated by serial X-rays. The use of adjunctive modalities to promote fusion, such as electrical bone stimulation, should be considered in this patient population as these patients and procedures are considered at high risk for nonunion.

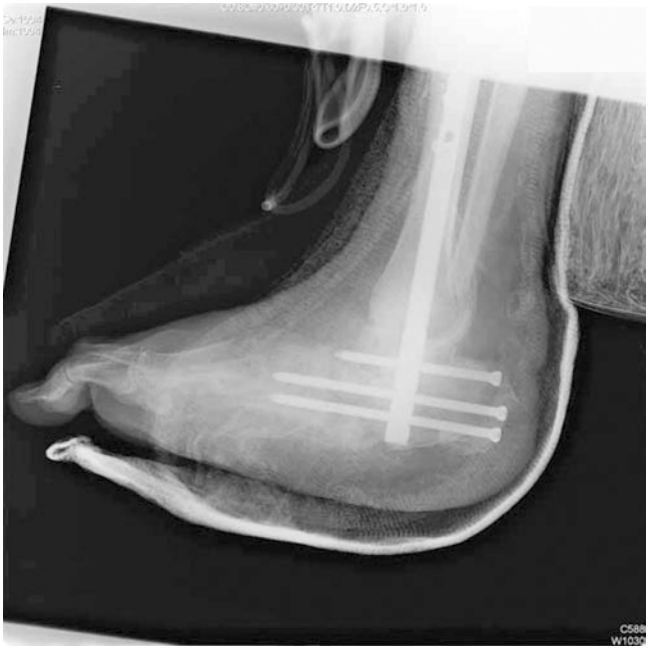

Fig. 19.21 X-ray showing Charcot ankle reconstruction using an intramedullary nail and femoral head allograft

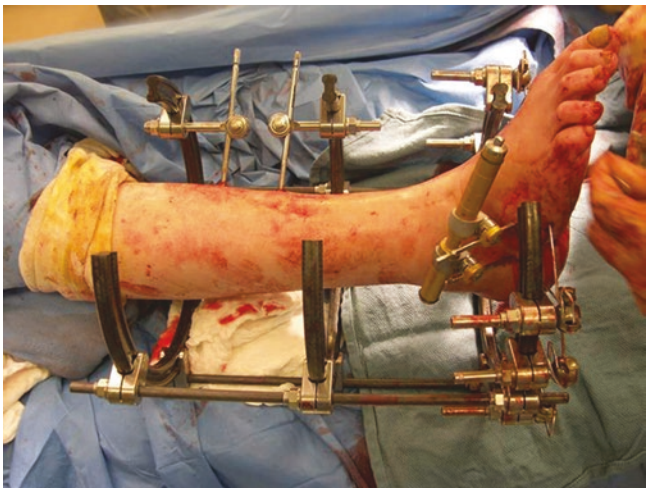

Fig. 19.22 Severe Charcot deformity with an open ulceration and osteomyelitis will require the use of external fixation to correct the deformity and to avoid the use of internal fixation at the site of ulceration and osteomyelitis

Arthrodesis with External Fixation

The complex nature of these deformities, open ulcerations with or without osteomyelitis, and significant bone loss have recently required utilization of recent advances in external fixation. Significant bone loss in these hindfoot deformities often do not allow for dependable use of internal fixation devices. In addition, the presence of an open ulceration and osteomyelitis makes the use of internal fixation contraindicated. Therefore the use of various external fixation constructs has been used to achieve stabilization in these deformities [70–73]. The most common construct utilizes a combination of multiplane fine wire ring fixators and half pins attached to the leg and foot at different levels. If possible, this can be used in conjunction with internal fixation (Figs. 19.22 and 19.23a, b).

Tendoachilles Lengthening

The effect of a tight Achilles tendon on foot mechanics, foot ulcerations, and Charcot joint disease has been well documented [74–76]. A tight Achilles tendon from enzymatic glycosylation leads to increased plantar foot pressures resulting in foot ulcerations. A tight Achilles tendon is also present in Charcot joint disease. The majority of clinicians caring for diabetic foot ulcerations agree that a tight Achilles tendon contributes to the recurrent nature of diabetic foot ulcerations and should be addressed via tendon lengthening.

The Achilles tendon is formed by the end fibers of the gastrocnemius and soleus muscles and insert into the dorsal posterior aspect of the calcaneus. By virtue of its insertion, the Achilles tendon functions as a strong plantarflexor at the ankle joint and invertor of the subtalar joint. Its plantar flexion motion is opposed by the extensor muscles crossing the anterior aspect of the ankle joint and its inversion motion is opposed by the peroneal muscles laterally. When the calcaneus everts and the axis of the subtalar joint changes as occurs in patients who excessively pronate, the axis of pull of the Achilles tendon also changes. It now creates a strong pronatory force of the foot. This can lead to excessive medial transfer of weight and midfoot collapse as seen in Charcot joint disease. It is for this reason that the Achilles tendon must be evaluated in every case of Charcot joint disease and reconstructive surgery. Failure to recognize this fact can lead to recurrence of the ulceration and failure of the reconstruction.

There are several techniques to lengthen the Achilles tendon [77–80]. These can be classified as either open or percutaneous. The simplest technique is the percutaneous approach (Fig. 19.24). This technique uses three small stab incisions and minimal soft tissue dissection. However, this requires an understanding of the anatomy of the Achilles tendon and the ability to convert to the open technique when necessary. The procedure can be approached with the patient lying either supine or prone. Three small stab incisions are made centrally on the Achilles tendon. The incisions are spaced approximately 1–1.5 cm apart with the distal most incision being 1.5 cm from the insertion of the Achilles on the calcaneus. The most proximal and most distal incisions will incise the Achilles centrally and exit laterally while the middle incision will incise the Achilles centrally and exit medially. Once

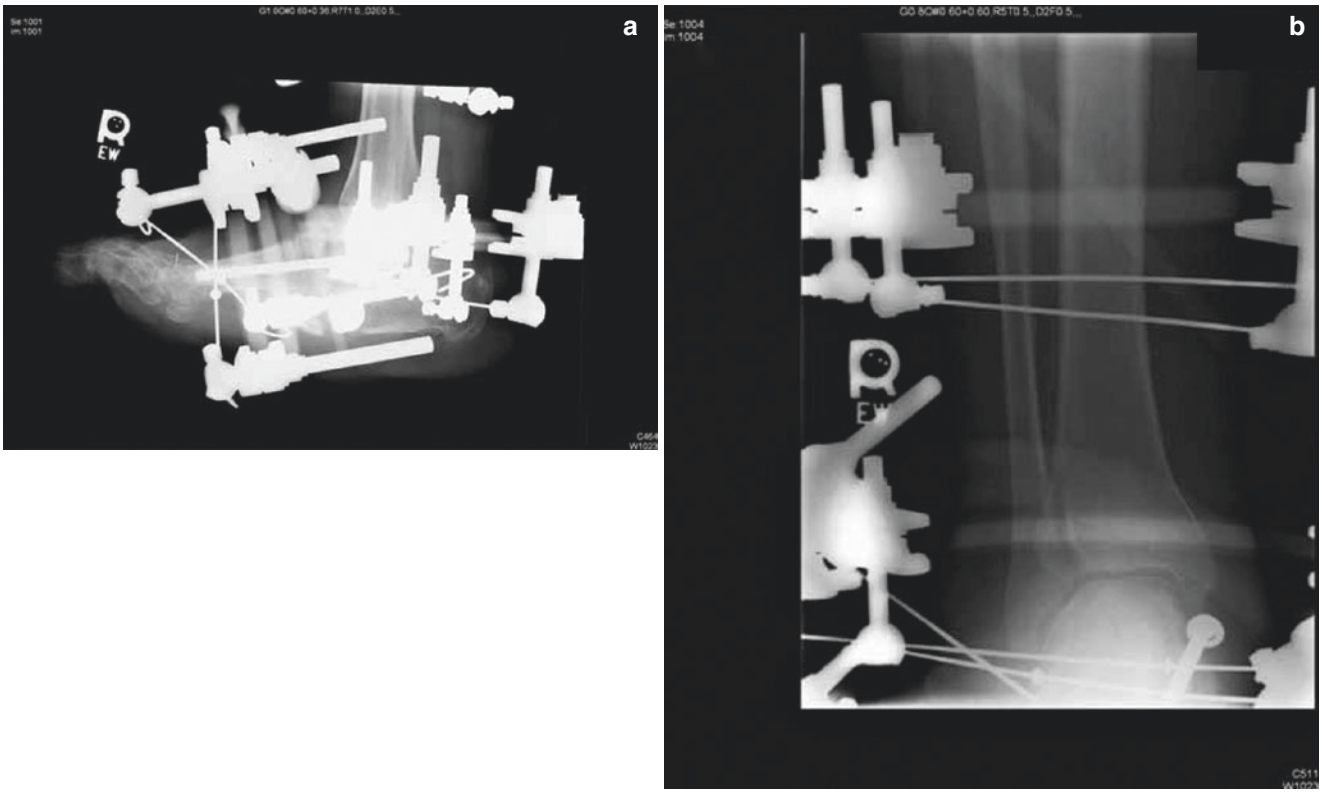

Fig. 19.23 (**a**) Charcot reconstruction demonstrating correction with combination of internal and external fixation to address midfoot and hindfoot deformities (*lateral view).* (**b**): External ring fixator using thin wire technique. (*AP view*)

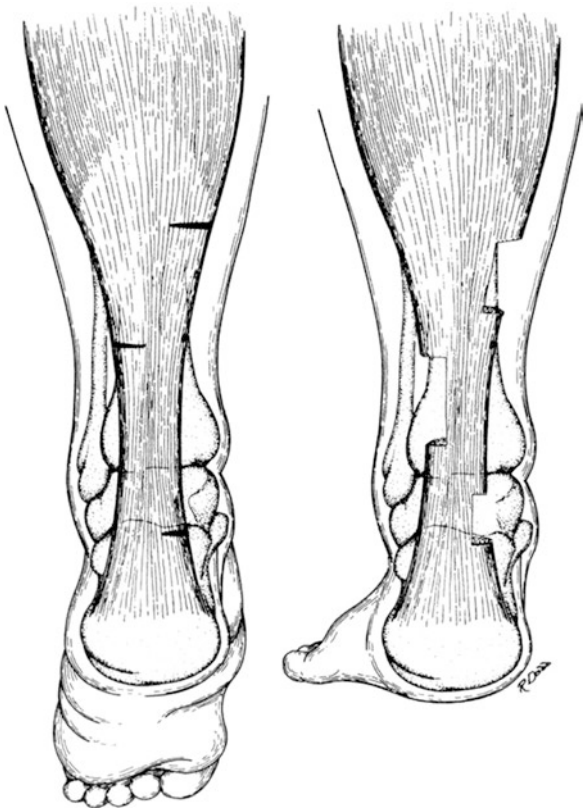

Fig. 19.24 The percutaneous technique uses two medial stab incisions and one lateral incision. The ankle is dorsiflexed to allow for lengthening of the Achilles tendon

the three incisions are completed, a gentle dorsiflexing force is exerted on the foot until a gentle stretch can be felt on the Achilles. Care should be taken not to stretch the Achilles beyond 10° of dorsiflexion. The skin incisions are then closed with suture of the surgeons' choice.

While the percutaneous technique provides adequate correction and is the least morbid technique, there are situations where greater degrees of correction are needed. This is where the open technique may be needed (Fig. 19.25). This is best performed with the patient prone. An approximately 8–10 cm incision is made along the central portion of the Achilles tendon. The incision is deepened until the peritenon is visualized. The peritenon is incised longitudinally along the line of the skin incision exposing the Achilles tendon. While there have been several ways described to lengthen the tendon, our preferred method is to make one incision approximately 1.0 cm proximal to the insertion. The blade is inserted into the midsubstance of the Achilles all the way across and the anterior fibers are transected. Attention is then directed approximately 2.5–3.0 cm proximally where the blade is once again inserted into the midsubstance of the Achilles tendon. The posterior fibers of the tendon are now transected. Once completed, the foot is once again gently dorsiflexed until the tendon can be seen to lengthen along the central intact fibers. In this fashion, the surgeon can visualize the amount of lengthening achieved and "dial-in" more dorsiflexion if necessary and if feasible. Closure of the wound,

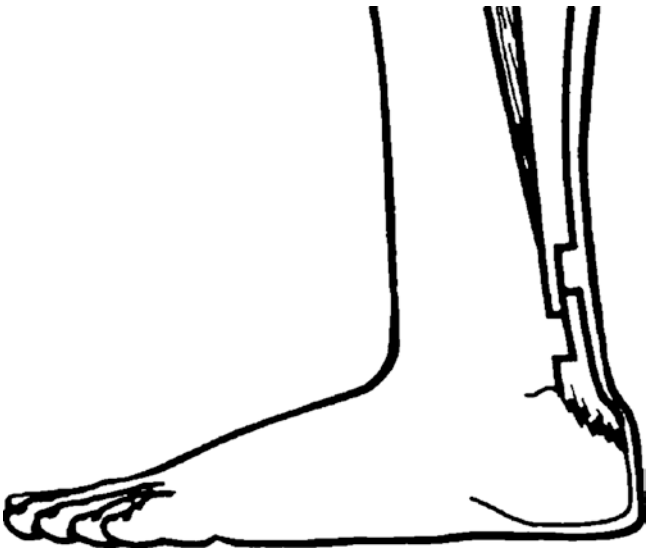

Fig. 19.25 The open Achilles tendon lengthening creates incisions in the tendon proximally and distally. The tendon is then lengthened in the frontal plane

including the peritenon, is performed in a layered fashion. The Achilles tendon lengthening is protected for approximately 6 weeks in a splint or brace that maintains the ankle joint at 90°.

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Lower Extremity Arterial Reconstruction in Patients with Diabetes Mellitus: Principles of Treatment

20

Douglas W. Jones and Mark C. Wyers

Abstract

In patients with peripheral arterial disease (PAD), optimizing the chances for successful revascularization requires careful consideration of several preoperative factors. PAD should be appropriately staged and any concurrent foot infection should be managed to obtain source control. Evaluation of fitness for revascularization can be assessed in a number of ways, focusing on frailty, cardiac disease, and renal insufficiency. Preoperative imaging may include CTA or MRA; however, diagnostic angiography is the most detailed, and is therefore essential in determining whether a patient is appropriate for any revascularization attempt. Angiography also guides the choice between open or endovascular approaches. The presence of an adequate autologous bypass conduit, determined by preoperative vein mapping, is also important in deciding which revascularization technique to pursue initially.

In the diabetic population, the infrapopliteal arteries are the most common site of occlusive disease. Endovascular techniques rely on crossing these areas and reopening them with balloon angioplasty. A successful procedure results in uninterrupted blood flow to the arteries of the foot. Balloon angioplasty alone is the standard modality of treatment in the infrapopliteal arteries. Bare metal stents, drug-coated balloons, and drug-eluting stents have been studied in this area, but despite promising results, they are not widely available.

Surgical bypass is most commonly performed for extensive, multilevel occlusive disease. Successful bypass requires a healthy inflow artery, a patent distal target artery with in-line flow to the foot, and a high-quality autogenous conduit (typically great saphenous vein). Arm vein bypass can also be performed with good outcomes. Prosthetic conduits are not typically used in bypass of infrapopliteal arteries as results are poor. The breadth of endovascular and surgical options for limb salvage in the diabetic patient has expanded to the point where treatment plans are highly individualized and combinations of techniques are common.

Introduction

Understanding the complex interplay of peripheral neuropathy, ischemia, and infection in the diabetic foot ulcer patient is essential to limb salvage. Lower extremity peripheral arterial disease (PAD) is one of the most significant factors contributing to major amputation in this population [1]. An important principle in the treatment of diabetic vascular disease is recognizing that the most common cause is *macro*-vascular atherosclerotic occlusive disease, usually involving the tibial arteries below the knee. The historic assumption that gangrene, nonhealing ulcers, and incomplete healing of minor amputations result from microvascular occlusion—so-called "small vessel disease"—has been refuted in multiple studies [2–7]. Unfortunately, this unsupported notion has resulted, for some, in a pessimistic attitude towards treatment of ischemia with resultant early amputation. Limb salvage and wound healing in diabetic foot ulcer patients is often possible but requires early vascular assessment and rigor in revascularization, ideally before advanced infection or tissue loss is present. The development of a more thorough understanding of PAD etiology and anatomic distribution has coincided with advances in minimally invasive, endovascular techniques, and refinements in the performance of

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surgical bypass. As a result, there are highly effective means of limb salvage in diabetic patients with arterial insufficiency that can be tailored to suit the individual situation.

Patient Selection

Peripheral Arterial Disease Staging

The presence and severity of PAD may be assessed in several ways. The simplest is through a detailed history and physical exam. The history may reveal symptoms of intermittent claudication, rest pain, or foot ulceration. A foot exam is performed to identify wounds or ulcers. Pedal pulses are palpated to assess distal perfusion. A combination of history with physical exam findings can lead to a basic classification of PAD as: asymptomatic, intermittent claudication or chronic limb-threatening ischemia (CLI). CLI is further classified as ischemic rest pain or foot ulcer.

The Rutherford classification system has been widely used to categorize symptomatic limb ischemia [8]. In this system, a score from 1 to 6 is assigned based on symptoms, physical findings, and hemodynamic parameters. The first three categories (Rutherford 1–3) describe patients with long, moderate, and short-distance claudication. The remaining categories describe patients with critical limb ischemia (CLI): category 4—ischemic rest pain, category 5—minor tissue loss, category 6—major tissue loss. Interestingly, hemodynamic parameters may not correlate with clinical symptoms. The Rutherford classification is useful for stratifying those patients (Rutherford 4–6) at much higher risk for amputation without revascularization. This original clinical categorization of CLI patients however, was intended for patients without diabetes, and makes no attempt to incorporate the complexity of concurrent infection and neuropathy commonly seen in diabetic patients [9].

In an effort to address the deficits of the Rutherford classification system, the Society for Vascular Surgery (SVS) has recently introduced the *W*ound, *I*schemia and *f*oot *I*nfection (WIfI) classification with the goal of providing a more comprehensive clinical staging system [9]. Similar to the TNM staging system for cancer, the WIfI system stages limbs based on three variables: (1) *W*ound extent (based on clinical findings), (2) *I*schemia (based on hemodynamic parameters such as ankle-brachial index, toe pressure or transcutaneous tissue oxygenation), and (3) severity of concurrent *f*oot *I*nfection (based on clinical findings of infection). Patients are scored within each category and then assigned a stage 1–4 with stage 4 representing the most severe limb-threatening ischemia (Table 20.1).

An important implication of the WIfI system is that as a patient's wounds heal or as their ischemic status is improved, their WIFi stage can change and this can be monitored.

Table 20.1 Wound, ischemia, and foot infection classification of peripheral arterial disease

Score	Wound	Ischemia	Foot infection
0	No ulcer No gangrene	ABI ≥ 0.80 Ankle pressure > 100 mm Hg TP ≥ 60 mm Hg	No symptoms or signs of infection
1	Small ulcer No gangrene	ABI 0.6–0.79 Ankle pressure 70–100 mm Hg TP 40–59 mm Hg	Local infection with ≤ 2 cm surrounding erythema
2	Deep ulcer with exposed bone/joint/tendon Or Gangrenous changes limited to digits	ABI 0.4–0.59 Ankle pressure 50–70 mm Hg TP 30–39 mm Hg	Local infection with > 2 cm surrounding erythema Or Involving structures deeper than skin
3	Extensive wound Or Extensive gangrene	ABI ≤ 0.39 Ankle pressure 30–39 mm Hg TP < 30 mm Hg	Local infection with signs of systemic inflammatory response syndrome (SIRS)

Conversely, worsening of a foot ulcer and infection would result in a more advanced WIfI stage that can be tracked over time. Such changes would not be identified in systems that focus only on amputation-free survival. In this way, the WIfI system allows for a more nuanced evaluation of outcomes as well as clinical staging. The WIfI system has recently been validated as a predictor of wound healing time and clinically relevant endpoints following endovascular intervention [10, 11] (Fig. 20.1).

An understanding of the WIfI system and its clinical relevance is important for risk stratification in patients with diabetic foot ulcers. The complexity of the system reflects the wide range of presentations in patients with PAD and diabetes. It is our experience that correction of hemodynamic abnormalities will only lead to successful limb salvage if there is appropriate wound care and control of infection. Multidisciplinary care, addressing all these aspects, is essential. If a patient with chronic limb-threatening ischemia is thought to be a candidate for limb salvage, revascularization is typically pursued following control of foot infection.

Control of Infection Prior to Revascularization

Patients with an unsalvageable foot due to medical comorbidities, preexisting nonambulatory status, and extensive necrosis from infection, or ischemia (i.e., advanced WIfI stage) may require primary limb amputation. However, in patients with an appropriate risk profile, proper control of active, spreading infection should be accomplished prior to

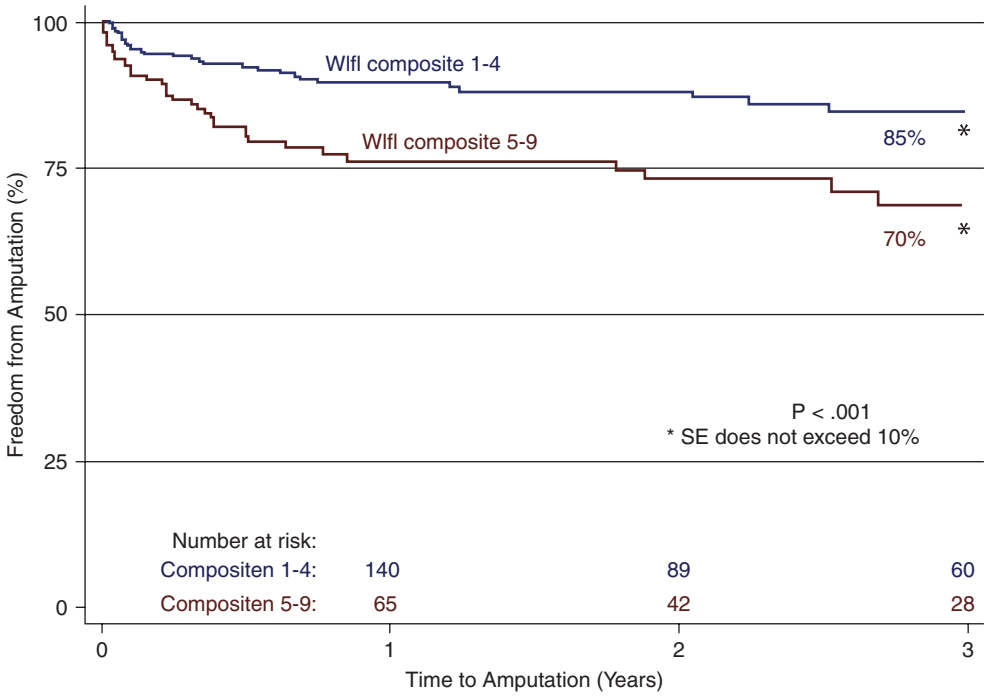

Fig. 20.1 WifI composite score predicts worse amputation-free survival following endovascular infrapopliteal intervention. From Darling JD, et al. *J Vasc Surg*. 2016;64(3):616–22

arterial intervention. Practically speaking, foot debridement is only given priority over revascularization in order to control wet gangrene, deep space abscess, or severe infection. Patients without symptoms or signs of local or systemic infection do not need to be started on antibiotics. In patients with infected wounds, antibiotics may be initiated based on guidelines formulated by the Infectious Disease Society of America (IDSA) and adapted by the SVS [9, 12]. Once culture data is available, antibiotic coverage can then be appropriately adjusted. In addition, those patients with abscess formation, septic arthritis, or necrotizing fasciitis should undergo prompt incision, drainage, and debridement including partial open toe, ray or forefoot amputation as indicated. [13] (Fig. 20.2). Such debridements are performed with the goal of preserving sufficient tissue to allow for later reconstruction and closure.

Preoperative Evaluation

Frailty

Certain patients such as those who are nonambulatory or bedridden, and have no likelihood of successful rehabilitation, may not be appropriate for arterial reconstruction. Similarly, patients with severe flexion contractures of the knee or hip are poor candidates for arterial reconstruction. Patients with end-stage diseases such as terminal cancer, those with very short life expectancy, or similarly lethal comorbidities have high complication rates with vascular reconstruction and may be better-served by primary amputation. And while patients with tissue loss and age >80 years are considered to be at high risk when pursuing surgical bypass [14], age alone is not a contraindication to arterial reconstruction. Frailty indices may be more sensitive indicators of a patient's physiologic age and therefore a better assessment of which elderly patients are more likely to benefit from revascularization. Different assessments of frailty have been developed but have yet to be widely adopted [15, 16].

Cardiac Disease

Patients with limb ischemia who present with active coronary artery disease (CAD) such as unstable or severe angina, decompensated congestive heart failure, significant arrhythmias or severe valvular disease may benefit from further cardiac evaluation prior to arterial bypass surgery [17–19]. When indicated, these patients typically undergo preoperative echocardiography with nuclear stress testing. Coronary angiography and percutaneous coronary intervention may also be necessary though additional antiplatelet medications may be required post-intervention which impact surgical timing and bleeding risk. In patients with active coronary artery disease, these interventions must be planned carefully. However, in the absence of active symptoms, a patient with stable coronary disease does not require preoperative cardiac workup prior to revascularization [17]. The exception is in patients with elevated cardiac risk and poor functional capacity (<4 MET equivalents) who may have unstable coronary artery disease without symptoms and therefore may benefit from preoperative cardiac testing and intervention [19]. In patients with high cardiac risk, endovascular interventions are thought to carry a lower risk of perioperative adverse cardiac event and, as a result, may be preferred.

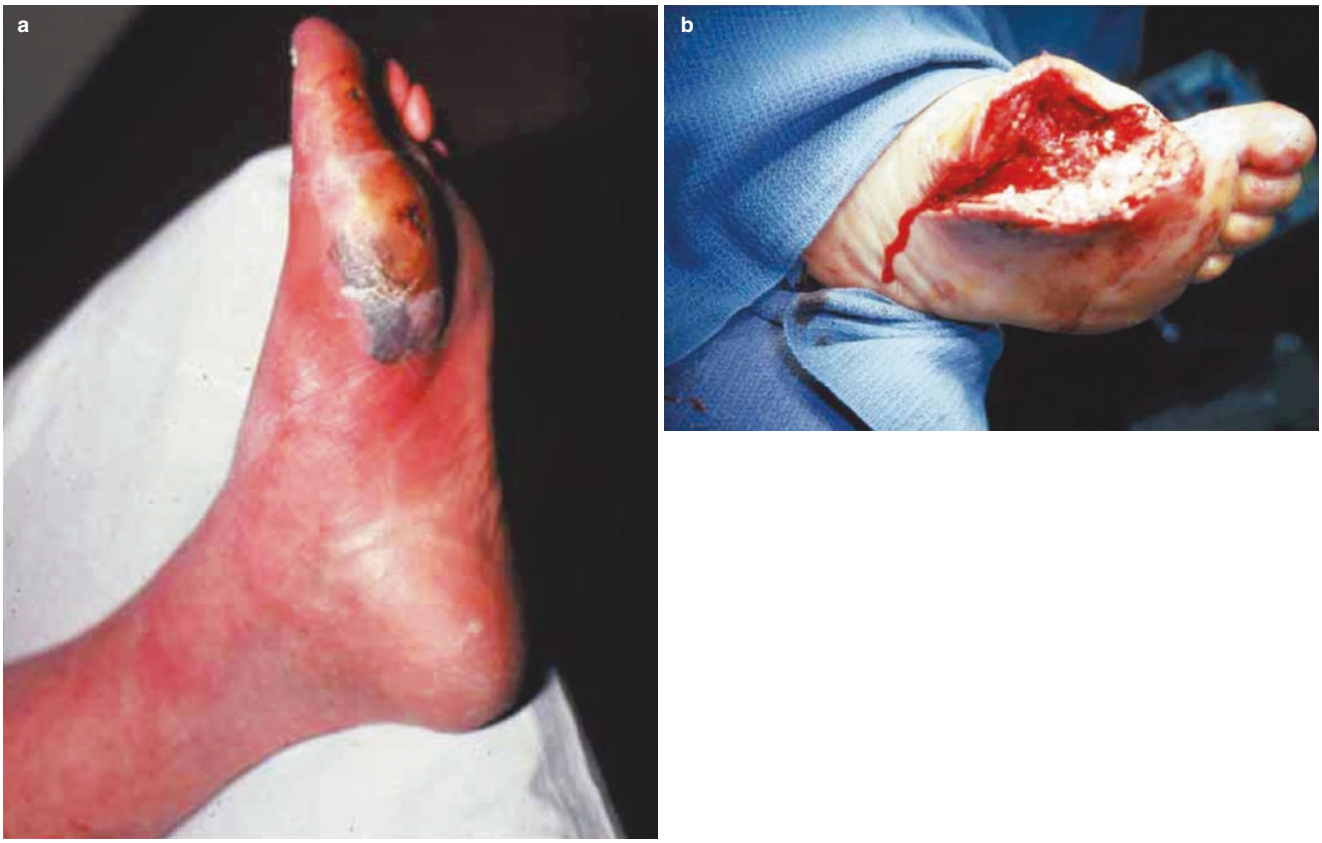

Fig. 20.2 Photographs of the right foot of a patient with diabetes who presented with a rapidly spreading infection as a result of a plantar ulcer over the first metatarsal. (**a**) Marked swelling and erythema of the medial forefoot are evident. There was palpable crepitus and malodorous drainage owing to involvement of the bone, joint, and flexor tendon with gas-forming bacteria. (**b**) Control of this infection required an emergent open first ray amputation. Cultures grew multiple organisms, including *Staphylococcus aureus*, *Proteus*, and anaerobes

Renal Insufficiency

Patients with limb ischemia in the setting of renal failure present particular challenges. Withholding or delaying contrast arteriography in patients with diabetes and compromised renal function is usually unnecessary. If there are extreme concerns about renal function, duplex ultrasound imaging, magnetic resonance angiography, and CO_2 or gadolinium angiography are imaging alternatives that can sometimes provide adequate information to plan arterial reconstruction or to allow for more limited and selective contrast arteriography of the tibial and pedal vessels [20]. When acute renal insufficiency develops, sometimes as a result of contrast-induced nephropathy after diagnostic angiography, surgery should be delayed until renal function stabilizes or returns to baseline. Most such patients will demonstrate a transient rise in serum creatinine in the absence of other symptoms. It is rare that such patients will become anuric or require hemodialysis.

Patients with chronic, dialysis-dependent renal failure (end-stage renal disease [ESRD]) can safely undergo arterial reconstruction. Many ESRD patients have severe, advanced atherosclerosis and have target arteries that are often heavily calcified. Gangrene and tissue loss are frequently present and the healing response in such patients is poor, even with restoration of pulsatile arterial blood flow to the foot. Some ESRD patients will require amputation even with patent arterial bypass grafts. Several studies have demonstrated that while reasonable, graft patency and limb salvage rates in these patients are lower than in patients without ESRD [21–24]. Our own study of 146 patients with ESRD undergoing arterial reconstruction for critical limb ischemia demonstrated graft patency and limb salvage rates of 68% and 80%, respectively, at 3 years. Perioperative mortality rate was reasonably low at 3%; however, long-term survival was poor with only 18% or patients alive after 3 years. Other studies have documented higher perioperative mortality rates (9–18%) and lower limb salvage rates (65–70% at 1 year) in this population. Despite this, revascularization is still a reasonable option for ESRD patients with critical limb ischemia rather than primary amputation in selected patients [25, 26]. Clinical judgment is paramount when considering arterial bypass for limb ischemia in the dialysis patient. Until further studies clarifying the role of bypass surgery in this patient population are available, treatment plans must be individualized.

Anatomic Imaging Prior to Revascularization

Vein Mapping

It is our preference to obtain bilateral lower extremity venous mapping in patients being considered for revascularization. Vein mapping entails duplex ultrasound evaluation of the great and small saphenous veins and provides information on patency, diameter, wall thickening, and intraluminal webs or thrombus. When leg vein is not available or is not suitable, arm vein mapping should be performed in search of an acceptable cephalic or basilic vein. Our preference is that the vein, whether saphenous or other, be larger than 3 mm in diameter and free of evidence of wall thickening. Vein mapping is typically performed with a gentle tourniquet placed on the proximal aspect of the extremity to dilate the vein distally.

CTA

While noninvasive vascular studies are excellent for determining which patients are likely to be candidates for revascularization, additional anatomic information may be required for operative planning. Computed tomography angiography (CTA) has become an important adjunct in this regard. In patients with PAD, CTA performs well, with sensitivity and specificity rates as high as 95% and 96%, respectively, for detection of >50% stenosis or occlusion [27, 28]. However, contrast administration is required which exposes patients to the risk of contrast-induced nephropathy. This risk is further exacerbated by coincidental renal insufficiency which is often associated with diabetes [29, 30]. Furthermore, CTA imaging can be compromised in patients with extensive vessel calcification, commonly seen in diabetic patients [31].

MRA

Magnetic resonance angiography (MRA) is typically performed with the use of intravenous gadolinium-based contrast agents. In this setting, sensitivity and specificity for detection of >50% stenosis or occlusion may be as high as 95% and 97%, respectively [32]. The advantages of MRA are that it is noninvasive and images are less likely to be degraded by vessel calcification [33]. The disadvantages are that gadolinium-based contrast exposure carries a small risk of nephrogenic systemic fibrosis (particularly in patients with chronic renal insufficiency) [34]. Additionally, patients with implanted devices, such as pacemakers, may not be able to undergo MRA.

Diagnostic Digital Subtraction Angiography

In our experience, arteriography is the best option for planning interventions because it provides the most anatomic detail and allows for immediate endovascular intervention when indicated [33]. The benefits of arteriography should be weighed against the risk of arterial access-related complications and, similar to CTA, iodinated contrast exposure. Using the techniques described below, however, we are able to use smaller volumes of contrast for diagnostic arteriography than the amount required for CTA in a majority of cases.

We routinely obtain arterial access using ultrasound guidance as it has been associated with decreased access-related complications [35, 36]. Contralateral, retrograde access is most commonly obtained using a 4-French sheath. A side-hole injection catheter is then advanced to the level of the L1-L2 vertebral interspace and an aortogram is performed using diluted contrast to delineate the anatomy of the abdominal aorta and its branches. A catheter is then advanced over the aortic bifurcation and unilateral lower extremity arteriography is obtained using digital subtraction techniques. It is crucial to visualize the entire tibial and pedal circulation since the former is the most common location of significant occlusive lesions in patients with diabetes and the latter is an important potential site for placement of the distal anastomosis when performing bypass surgery. Thorough delineation of vascular anatomy of the foot requires both a lateral and anterior-posterior view (Fig. 20.3).

As previously discussed, acute renal failure is a concern in diabetic patients undergoing contrast arteriography, especially in those with preexisting renal insufficiency. When renal failure does occur, it is almost always reversible, but may delay arterial reconstruction surgery for several days while the creatinine returns to baseline [37, 38]. Arteriography and percutaneous interventions may be performed safely even in patients with baseline renal insufficiency by following several basic precautions. CO_2 angiography may be used to image the aortoiliac and femoropopliteal segments but is usually not adequate for tibial artery anatomy (Fig. 20.4). Iodinated contrast may be diluted with saline to limit the amount used (usually to 1/2 or 1/3 strength). This dilute contrast can be used to perform focused angiography of the tibial and pedal stations. Dilute gadolinium can also be administered in small volumes but should be used cautiously due to risk of nephrogenic systemic fibrosis [34, 39]. With these considerations in mind, arteriography need not be withheld due to fear of exacerbating moderate chronic renal insufficiency.

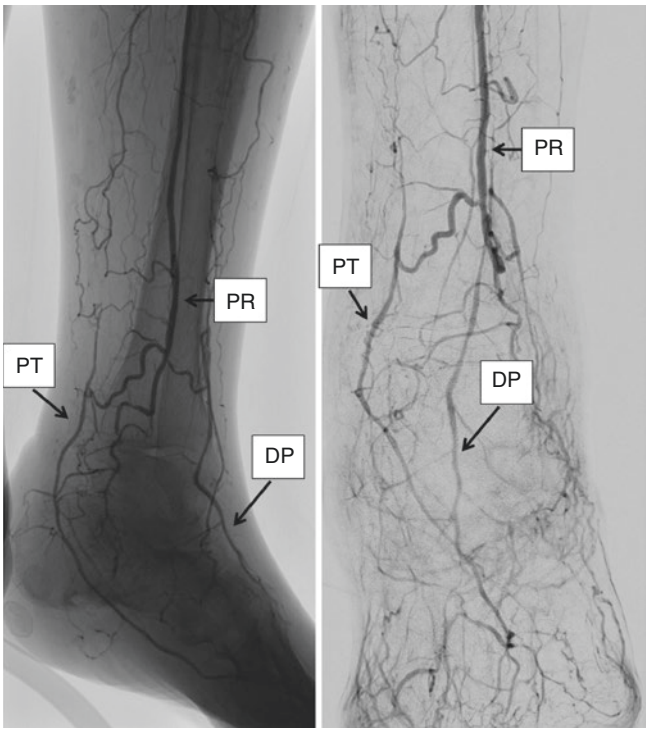

Fig. 20.3 Unsubtracted, lateral view of the right foot showing a patient peroneal artery (PR) with filling of the posterior tibial artery (PT) via collaterals from the peroneal. The distal anterior tibial artery is also reconstituted from peroneal artery collaterals with outflow into a patent dorsalis pedis (DP)

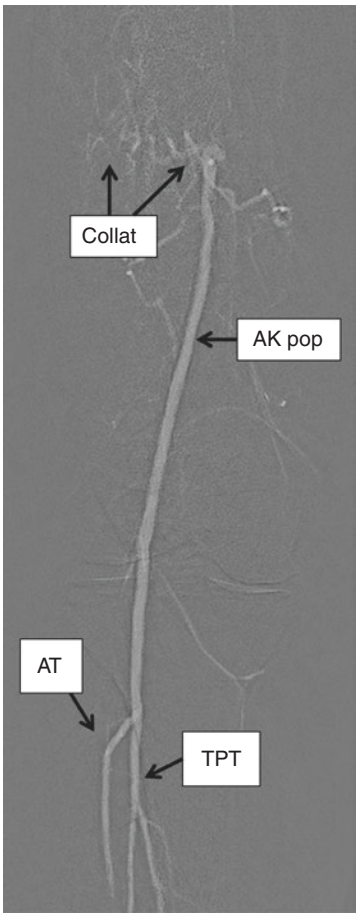

Fig. 20.4 CO_2 angiogram showing occluded distal right SFA with collateral filling (collat) of above-knee popliteal artery (AK pop). Good outflow into anterior tibial artery (AT) and tibioperoneal trunk (TPT). Posterior tibial artery and peroneal artery are also visualized and patent

Endovascular Revascularization

Technique

After diagnostic angiographic images are obtained, a plan for revascularization can be developed. Generally, the findings can be divided into four categories: (1) occlusive disease burden is not clinically significant and no intervention is required; (2) disease burden is extensive and not amenable to revascularization; (3) endovascular intervention is most appropriate; and (4) surgical revascularization is most appropriate.

The ultimate goal of lower extremity revascularization is to restore sufficient perfusion for ulcer healing. Generally, short stenoses or occlusions are suitable for endovascular techniques while long segment disease is best treated with surgical bypass. These recommendations have been formalized by the anatomic Trans-Atlantic Inter-Society Consensus (TASC) classification system [40, 41]. Though limited, this anatomic grading system provides a framework for determining which atherosclerotic lesions would be most appropriate for endovascular versus surgical intervention as a primary treatment. However, as previously discussed, this decision is complex and cannot be made in the absence of detailed understanding of a patient's clinical disease severity, including the extent of the wound, presence or absence of infection, and degree of ischemia. Detailed knowledge of available autogenous vein conduit is also helpful when deciding which treatment modality to pursue.

If the decision is made to proceed with endovascular intervention, the 4-French sheath is exchanged for a longer (45–90 cm) 5- or 6-French sheath that provides stable access over the bifurcation and into the leg of interest. Intravenous heparin is infused as a bolus in a dose of 80–100 units/kg and an activated clotting time (ACT) is checked periodically. We routinely maintain an ACT greater than 250 s for aortoiliac and femoropopliteal interventions, or greater than 300 s for tibial interventions. Once fully anticoagulated, various wires and supporting catheters can be utilized in a coaxial fashion to cross stenoses or occlusions. Every effort is made to keep the wire within the vessel lumen when crossing a lesion. However, it is sometimes necessary to

cross an occluded vessel in a subintimal plane and reenter distally at a site of less diseased artery. Short stenoses may respond well to balloon angioplasty alone (Figs. 20.5 and 20.6). In the aortoiliac and femoropopliteal segments, long, calcified stenoses or occlusions are likely to have residual luminal compromise even after balloon angioplasty and may require stent placement. Long-segment occlusions of tibial vessels may also respond well to balloon angioplasty alone (Fig. 20.7). Angiography is repeated following angioplasty or stenting to evaluate the success of the intervention and to assess for complications including dissection and distal embolization. If detected, such complications may require additional endovascular maneuvers or systemic anticoagulation to resolve.

In some cases, it may be impossible to cross an infrapopliteal stenosis or occlusion from an antegrade approach (either from ipsilateral or contralateral femoral access) but there may be a patent distal tibial or pedal vessel that reconstitutes via collateral flow on angiography. Though this anatomic pattern of disease has typically been best served with bypass surgery, some patients may not be surgical candidates due to comorbid conditions or lack of appropriate conduit. In these cases, retrograde pedal artery access may allow for endovascular treatment of previously un-crossable tibial artery occlusions. In our experience, this technique is needed infrequently, after standard endovascular approaches have been attempted, and only in cases where bypass surgery is not a suitable option.

Outcomes

The Bypass versus Angioplasty for Severe Ischaemia of the Leg (BASIL) trial is the most important randomized, multicenter, prospective trial comparing angioplasty to bypass for critical limb ischemia due to infrainguinal arterial occlusive disease [42–44]. Though the endovascular techniques utilized in the percutaneous transluminal angioplasty (PTA) arm are now outdated, at 2 years follow-up, mortality, limb salvage, and survival were identical for the two groups. The cost of bypass was found to be higher but the PTA group required reintervention more frequently. Surprisingly, functional outcomes and quality of life measures were identical for both groups. In a post hoc analysis of patients surviving beyond 2 years, limb salvage and survival were higher for patients undergoing bypass. In spite of its shortcomings, this study validated the use of tibial angioplasty for critical limb ischemia especially for those patients with strong contraindications to surgery or anesthesia and with an anticipated life expectancy of less than 2 years.

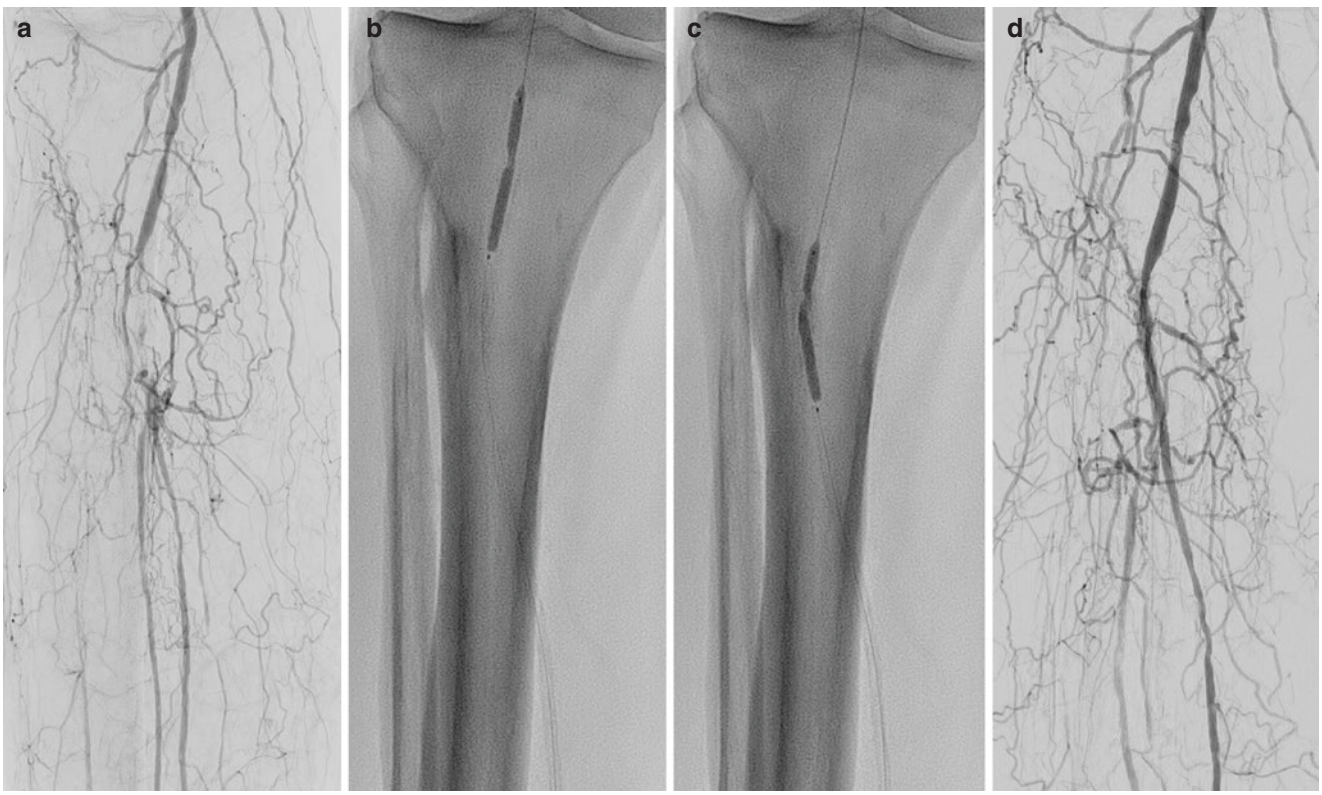

Fig. 20.5 (**a**) Pretreatment angiogram showing occlusion of right tibioperoneal trunk (TPT) and three tibial vessels. (**b**) Balloon angioplasty of TPT stenosis. (**c**) Balloon angioplasty of posterior tibial artery origin stenosis. (**d**) Completion angiogram showing restoration of in-line flow to the posterior tibial artery

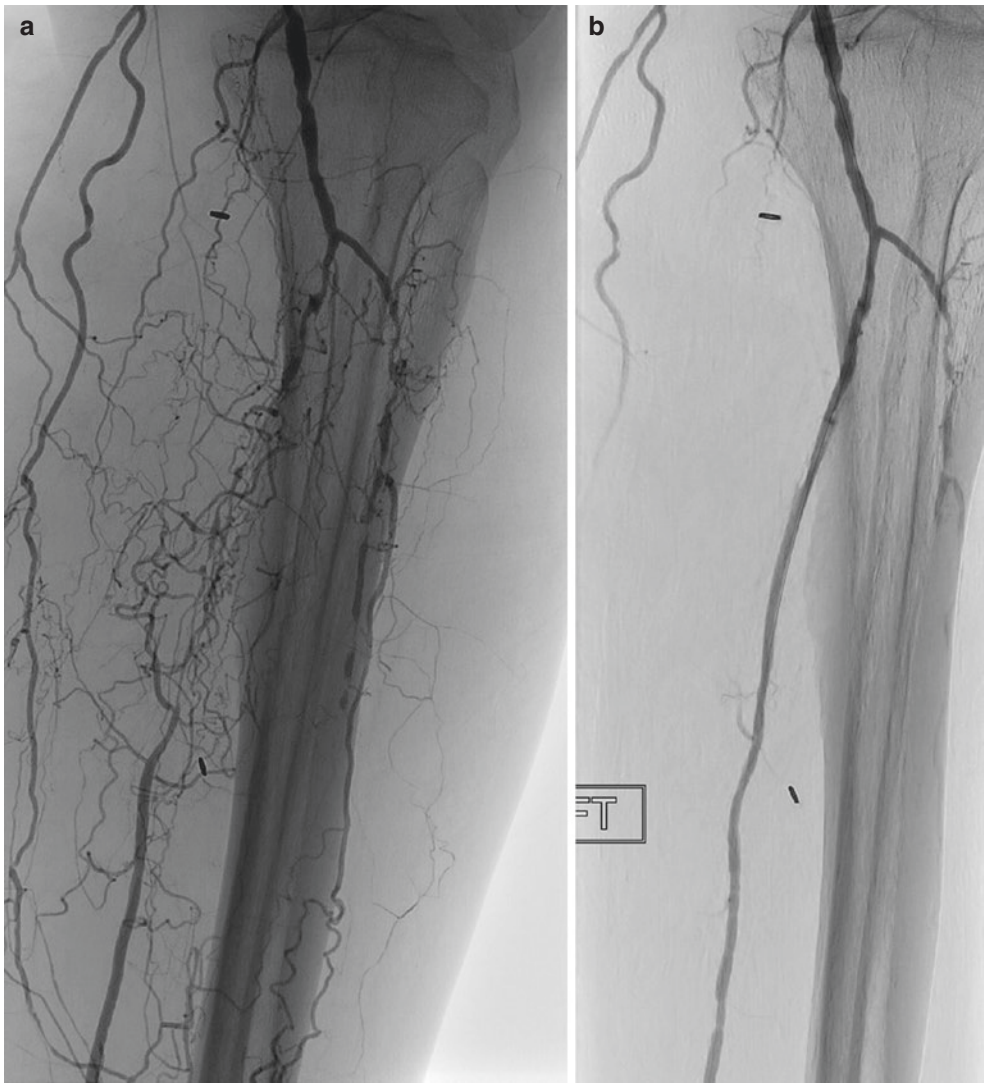

Fig. 20.6 (**a**) Pretreatment angiogram showing long-segment left posterior tibial artery occlusion. (**b**) Completion angiogram showing patent posterior tibial artery following angioplasty. Wide collateral network fills less robustly now that in-line flow has been re-established in PT

In our initial published series of infrapopliteal angioplasty in 176 limbs with CLI, technical success was achieved in 93% of patients overall and was noted to be related to lesion length: it was 100% for short, focal stenoses (1–4 cm) or occlusions (<2 cm) but decreased to 75% for longer occlusions (>2 cm) or diffusely diseased arteries. Patency of the treated vessel at 1 year was only 39% but limb salvage was 84% [45].

Following this report, we updated our institutional experience performing infrapopliteal angioplasty for patients with CLI [46]. Over an 8-year period, infrapopliteal PTA was performed in 459 limbs (average age 71 years). Of the 413 patients treated, comorbid diabetes was present in 75%. Technical success (residual stenosis <30%) was achieved in 93% of limbs. The 30-day mortality rate was found to be 6% and when only surgical candidates were considered, the 30-day mortality was slightly lower at 4%. In long-term follow-up, survival at 1, 3, and 5 years was 83%, 64%, and 49%, respectively (Fig. 20.8). Diabetes was not found to be an independent predictor of perioperative or long-term mortality. At 1-year follow-up, primary patency was 57% and limb salvage was 84%, and at 5 years follow-up, primary patency was 34% and limb salvage was 81%. Restenosis rate at 5 years was found to be 74%. Perhaps not surprisingly, worse outcomes were associated with more advanced occlusive disease, as indicated by TASC I classification [47], a finding which has been shown in other small series as well [48, 49]. The incongruity between excellent limb salvage rates and high restenosis rates is partially explained by the frequency and presumed efficacy of reintervention. Of all patients treated, 50% required repeat PTA and/or bypass at 5 years follow-up. These findings reinforce the need for continued surveillance and likelihood of repeat interventions in patients undergoing infrapopliteal angioplasty. The mortality rate of 4% in patients who are also surgical candidates challenges the notion that endovascular tibial interventions are safer than surgical bypass. For context, in our institutional report on 1000 cases of dorsalis pedis bypass

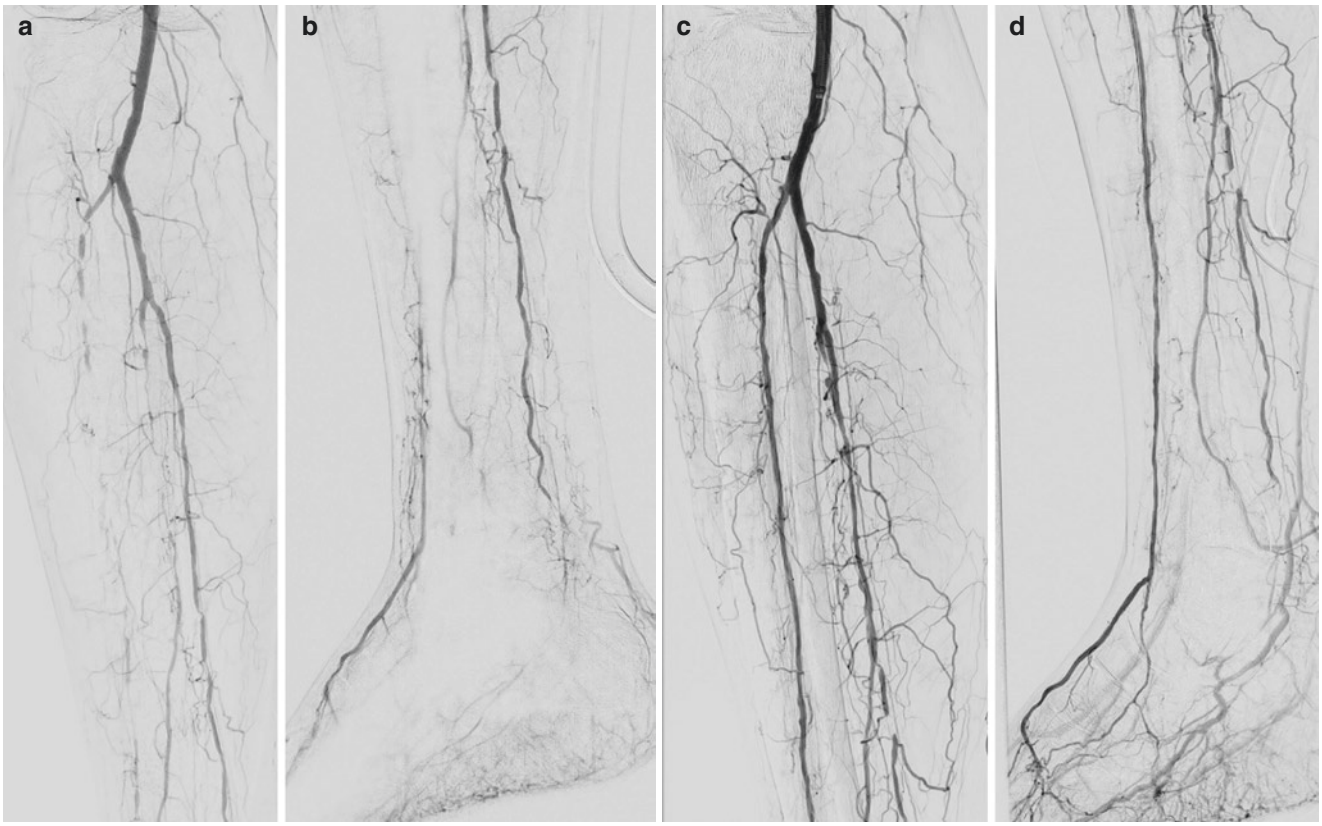

Fig. 20.7 (**a**, **b**) Subtracted pretreatment angiogram showing long segment occlusion of right anterior tibial artery with reconstitution of dorsalis pedis. (**c**, **d**) Subtracted angiogram showing patent AT following balloon angioplasty

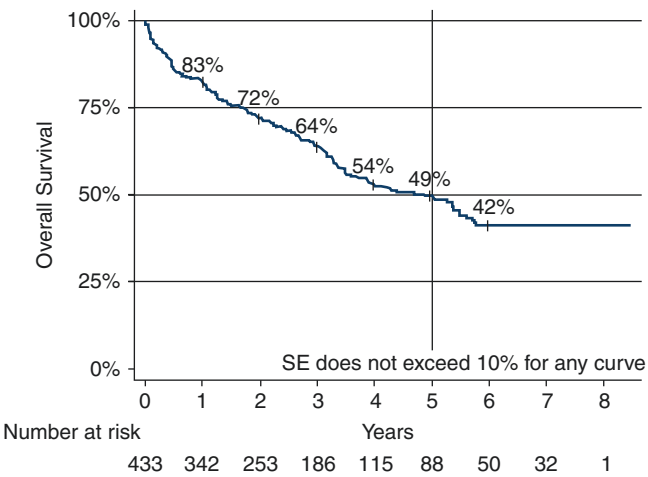

Fig. 20.8 Long-term survival following infrapopliteal angioplasty. Adapted from Lo, et al. *J Vasc Surg*. 2013;57(6):1455–63

(average age 66.8 years), a 30-day mortality rate of 0.9% was reported [50].

Others have reported their experience with infrapopliteal angioplasty as well. In a meta-analysis of infrapopliteal angioplasty for critical limb ischemia, more than 2500 patients were included, of which 61% had diabetes [51]. Technical success rate was estimated to be 89% and primary patency rates were 77% and 49% at 1 and 3 years, respectively. Similar to our reported experience, limb salvage rates were 93% at 1 year and 82% at 3 years indicating acceptable limb salvage rates in the setting of frequent restenosis.

For comparison, the authors also performed a meta-analysis of popliteal-dorsalis pedis bypass [52] and found that the limb salvage rate was comparable at 3 years (82.3% bypass vs. 82.4% PTA). Based on the results of the BASIL trial, 3 years of follow-up should be adequate to show differences between treatment modalities. These non-randomized results are encouraging, and suggest that endovascular interventions for infrapopliteal occlusive disease may be comparable to bypass in some patients.

Importantly, adjunctive technologies including drug-coated balloon angioplasty and drug-eluting stents have developed considerably in recent years [53]. Initial enthusiasm for drug-coated balloon (DCB) angioplasty in infrapopliteal arteries has diminished as the results of the INPACT-DEEP trial have become available [54]. This is the largest randomized trial of DCB versus PTA and showed no additional benefit to DCB. In fact, in the INPACT-DEEP study, DCB was associated with a trend towards higher rates of major amputation compared to PTA. There is currently no

compelling evidence that DCB has any additional benefit compared to PTA in this setting [53, 55].

Promising results regarding the use of drug-eluting stents (DES) have been more uniform. Four RCTs using drug-coated drug-eluting stents in the superficial femoral and proximal popliteal arteries have shown excellent primary patency for DES at 1-year follow-up [56–60]. Taken together, these studies have shown that DES appears to demonstrate clinically relevant improvements in patency, reduced reintervention rates and reduced amputation rates over PTA and bare metal stenting [61–65]. However, longer and more complex stenoses and occlusions may not be amenable to extensive stenting and, as our own experience has shown, these are the lesion types that are prone to failure with endovascular therapy. As DES becomes more widely available for use, the anatomic situations in which DES deployment is most useful should be carefully considered. In order to better describe when to pursue endovascular or surgical revascularization as the primary intervention, the BEST-CLI (Best Endovascular versus Best Surgical Therapy for Critical Limb Ischemia) trial was initiated [66]. Enrolled patients must be candidates for endovascular or surgical intervention and will be randomized between treatment types. In order to account for the importance of adequate autogenous conduit, patients with single-segment great saphenous vein will be randomized separately from patients with suboptimal autogenous conduit. The investigators seek to enroll >2000 patients and the results of this study are sure to have a lasting impact on operative planning in CLI.

Surgical Revascularization

Technique

A patient deemed to be a good candidate for surgical bypass must have an adequate inflow source and outflow target artery, both of which are typically determined using diagnostic DSA. The outflow target artery at the location of the distal anastomosis should be relatively free of occlusive disease and demonstrate unimpeded arterial flow into the arteries of the foot. In general the most proximal artery distal to an occlusion meeting these two criteria is chosen as a bypass target vessel. Distal arterial reconstructions present special technical challenges for the vascular surgeon and require meticulous attention to detail (Fig. 20.9). The target arteries are usually small, approximately 2.0 mm in diameter, and often affected by medial calcification.

Vein bypass grafts for chronic limb-threatening ischemia have well-established safety and efficacy. Perioperative mortality in most contemporary series ranges from 1 to 5% [67] and limb salvage may approach 90% at 5 years for many patients [68]. Although these results are excellent, they do not reflect the high cost of recovery for many patients to achieve this outcome. Wound morbidity is common ranging from 10 to 50% [69, 70]. Limb swelling, delayed healing of ischemic wounds, and the need for additional procedures may delay full recovery for many months. In our study evaluating quality of life measures in patients undergoing arterial bypass for limb salvage, less than 50% reported feeling they were back to normal 6 months after surgery [71]. In a similar study only 15% of patients achieved the ideal outcome of a patent graft with no need for revision, no wound complications, and a healed foot following bypass. These observations are especially sobering when considering the fact that 50% of patients survive less than 5 years after their limb salvage procedure [72].

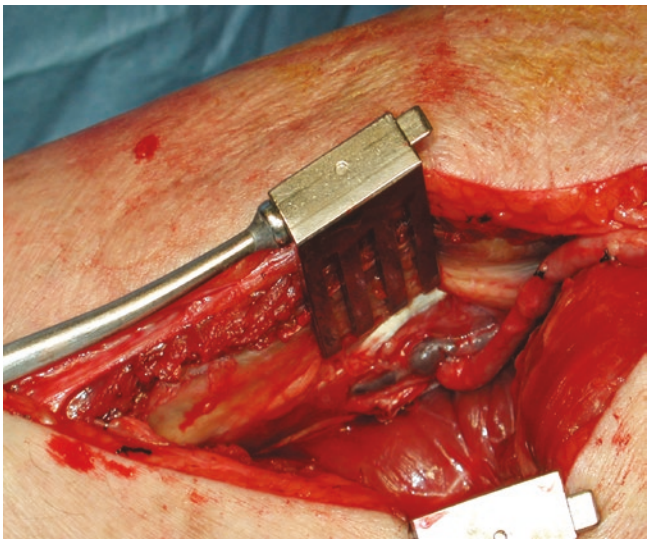

Fig. 20.9 Peroneal anastomosis in a common femoral-peroneal bypass using non-reversed greater saphenous vein. The distended saphenous vein graft with ligated vein branches is shown as it turns to meet the peroneal artery, deep in the lower leg, beneath a peroneal vein. Courtesy of David Campbell, MD

One of the most important developments in vascular surgery has been the demonstration that autogenous saphenous vein, as opposed to prosthetic graft material, gives the best short- and long-term results for distal bypass. In a large multicenter prospective randomized clinical trial, 6-year patency of saphenous vein grafts was more than four times higher than that of prosthetic grafts [73]. For over six decades, the standard graft orientation performed for lower extremity arterial revascularization has been the reversed saphenous vein bypass. The vein is completely harvested and its distal end is translocated to the proximal anastomotic site in order to prevent impediment of flow from intact venous valves. For distal bypass especially, this often creates a size discrepancy between the venous conduit and the inflow and target arterial anastomoses. To avoid this problem and minimize vein harvest trauma, valvulotomy was developed to render the valves incompetent. This allows the vein to be used in

either the traditional non-reversed or the more recent "in situ" configuration. In the late 1970s, Leather and associates popularized this technique using a modified Mills valvulotome that cuts the valves atraumatically to render them incompetent [74]. Vascular surgeons enthusiastically embraced the Leather technique and began reporting improved results with the non-reversed, in situ bypass compared with the conventional reversed vein approach [75–77]. This led some to conclude that the in situ bypass possesses inherent biologic superiority to the reversed saphenous vein graft [78]. However, further evidence to support this concept has not been presented [79]. Moreover, when in situ bypasses are compared to more contemporary series of reversed saphenous vein bypasses, no superiority is evident [80]. In our own experience, we have frequently used both procedures and have observed similar results with either vein configuration [81]. We also routinely perform angioscopy to perform valvulotomy under direct vision and evaluate the general quality of the vein.

In the 1980s Ascher and associates reported the first series of bypass grafts with inflow taken from the popliteal artery [82]. Because atherosclerotic occlusive disease often spares the superficial femoral artery in diabetes, the popliteal artery can be readily used as a source of inflow for the bypass graft. Doing so shortens the operative procedure time, shortens the length of the bypass, and avoids potentially troublesome groin wound complications, which often accompany thigh and groin dissections. Short vein grafts are also advantageous in patients who have a limited quantity of adequate saphenous vein. They showed results that were equivalent to those of the traditional approach that preferentially used the common femoral artery. Such results have been confirmed by other groups and this technique has proven to be another important advancement in arterial reconstruction for patients with diabetes [83, 84]. Our experience with extreme distal arterial reconstructions has shown that popliteal artery inflow is possible in about 60% of diabetic patients undergoing vascular reconstruction in the lower extremity [81].

Ipsilateral, single-segment great saphenous vein is the conduit of choice for infrainguinal leg bypass. When the ipsilateral saphenous vein is unavailable due to varicosities, previous harvesting, or stripping, alternative sources of conduit must be used. Although some surgeons use prosthetic grafts in these circumstances, alternative vein grafts including contralateral great saphenous vein, arm vein, or small saphenous vein can be used. In patients with an absent ipsilateral great saphenous vein, the likelihood of requiring another arterial reconstruction in the opposite extremity approaches 40% at 3 years following the first operation [85]. Because of this, some surgeons hesitate to harvest the contralateral saphenous vein even though it remains the better option [86].

When saphenous vein is unavailable bilaterally, our vein conduit of choice is cephalic or basilic vein. Our results with arm vein grafts have been improved by examining the vein with intraoperative angioscopy to exclude segments with strictures or scarring from trauma commonly induced by previous venipuncture or thrombosis [87]. Using the angioscope to evaluate the quality of arm vein conduit has significantly improved our results and further reduces the number of patients requiring prosthetic conduit [88]. Although arm vein is a reasonable conduit in most patients, we hesitate to harvest arm vein for leg bypass in patients with end-stage renal disease given the need potential arteriovenous access surgery. One potential disadvantage of arm vein conduits is their limited length. The use of popliteal artery inflow makes the use of shorter arm vein grafts possible in many patients. Moreover, in carefully selected patients, the use of composite grafts made by combining vein segments can provide enough conduit length to reach from the groin to the distal tibial and even foot vessels in many patients [89]. Our results with arm vein grafts in over 500 procedures have been reported [90]. Patency was 57.5% and limb salvage was 71.5% at 5 years. These results were inferior to those with reconstructions done with saphenous vein, however significantly better than those reported with prosthetic conduits.

A recent report on the outcomes of tibial bypass with prosthetic graft, heparin bonded polyfluorotetraethylene (ePTFE), versus saphenous vein conduit noted a significant improvement in patency rates associated with saphenous vein use. Patency of the graft was 75% with ePTFE versus 86% with saphenous vein graft during the follow-up period that ranged from 1 to 12 months [91]. This demonstrates the early patency advantage of using saphenous vein when available despite the advances in prosthetic graft construction.

In general, the goal of treatment is to restore maximal arterial flow to the foot since this provides the best chance for healing. The preoperative diagnostic arteriogram is the key piece of information necessary in planning the appropriate surgical procedure for each patient. If a bypass to the popliteal or tibial artery will restore maximal arterial flow and restoration of palpable foot pulses, bypasses need not extend to the level of the foot. Since the quality of venous conduit is the most important determinant in long-term success, using the shortest length of high-quality venous conduit necessary to achieve this goal is the basic rule. Each operation must be individualized based on the patient's available venous conduit and arterial anatomy.

Outcomes

The independent effect of diabetes on outcomes in patients undergoing surgical bypass is controversial and continues to be debated. In the PREVENT III trial, 1404 patients

underwent bypass for CLI, of which 64% had diabetes [92]. The authors found that diabetes status did not affect graft patency. Congruent with these results was the finding that when optimal vein conduit was used, there were no significant differences in patency between femoropopliteal bypasses and distal bypasses (to tibial or pedal vessels) [92, 93]. Our institutional experience with greater than 800 lower extremity bypass procedures has also shown no independent effect of comorbid diabetes on outcomes [94] (Fig. 20.10).

Findings from PREVENT III, CICRULASE, and BASIL trials were combined by the Society for Vascular Surgery to formulate objective performance goals for bypass surgery [14]. This analysis included only the highest quality randomized, controlled data of patients undergoing bypass with autogenous vein. The investigators found that older patients (age > 80) and patients with tissue loss should be considered "clinical high risk" due to demonstrably worse outcomes at 1-year follow-up. Diabetes was not determined to significantly impact 1-year outcomes and was therefore not included as a predictor of increased clinical risk. Despite the isolated PREVENT III findings showing comparable results for distal bypass, patients with infrapopliteal targets or who lack high-quality venous conduit should be considered "anatomic high risk." This anatomic risk classification arose from the observation that patients undergoing infrapopliteal bypass had lower rates of freedom from major adverse limb events (MALE) (74 vs. 81%, $p = 0.004$). These events include above ankle amputation or major reintervention at 1-year follow-up or perioperative death. However, there were no differences in mortality or amputation-free survival at 1-year follow-up based on anatomic risk criteria. Taken together, these data suggest that diabetes is unlikely to impart an independent risk of worse outcomes following bypass surgery. Distal bypass is very common in diabetics and is a highly technically challenging surgical procedure. As a result, there is a moderately increased risk of complications in patients undergoing distal bypass, but no difference in limb salvage or mortality.

Technical precision in the performance of tibial and pedal bypass in the diabetic population is absolutely essential to success. A review of arteriograms at our institution imaging the entire lower extremity circulation in patients evaluated for revascularization demonstrated that in 10% of cases a foot vessel, usually the dorsalis pedis artery is the only suitable outflow. In another 15% of patients, the dorsalis pedis artery appears to be a better quality outflow target vessel than other patent but diseased tibial vessels. As a result, we began performing bypasses to the dorsalis pedis artery for limb preservation in situations where no other (more proximal) bypass option existed [95]. We have reported our experience with vein bypass grafts to the dorsalis pedis artery in excess of 1000 procedures with follow-up extending beyond 10 years [50]. At 5 years, graft patency was 63% and limb salvage was 78%, however patient survival was less than 50%. Approximately 60% of patients requiring pedal bypass present with some degree of foot infection, and this raises concerns about placing an arterial graft in such close proximity to infected tissues. This, however, has not proved hazardous provided that active, spreading sepsis is controlled prior to surgery [96]. Our results have compared favorably with other reports of pedal level arterial reconstruction [97–101] and are comparable to or better than results now routinely reported for popliteal and tibial artery reconstructions.

In advanced cases of distal ischemia, or in cases of failed pedal bypass, patients may have no available outflow vessel other than the lateral tarsal branch of the dorsalis pedis artery or the lateral or medial plantar branches of the posterior tibial artery (Fig. 20.11). In our series of 98 tarsal and plantar

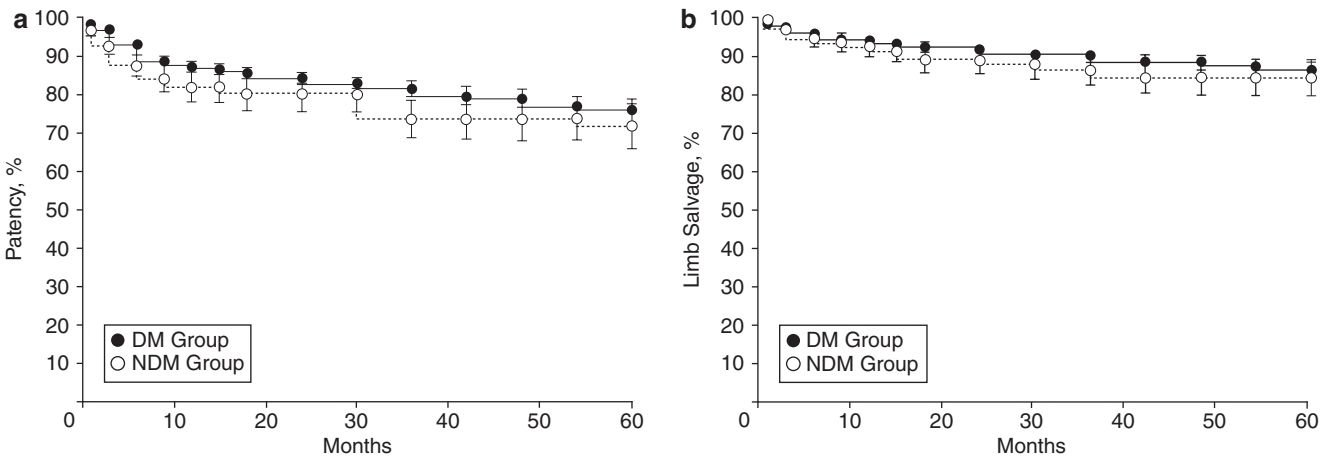

Fig. 20.10 Graft patency (**a**) and limb salvage (**b**) for diabetic and nondiabetic patients undergoing lower extremity bypass and followed for at least 5 years. *From Akbari CM, Pomposelli FB, Jr., Gibbons GW, Campbell DR, Pulling MC, Mydlarz D, LoGerfo FW. Lower extremity revascularization in diabetes: late observations. Arch Surg 2000;135:452–6* (*with permission*)

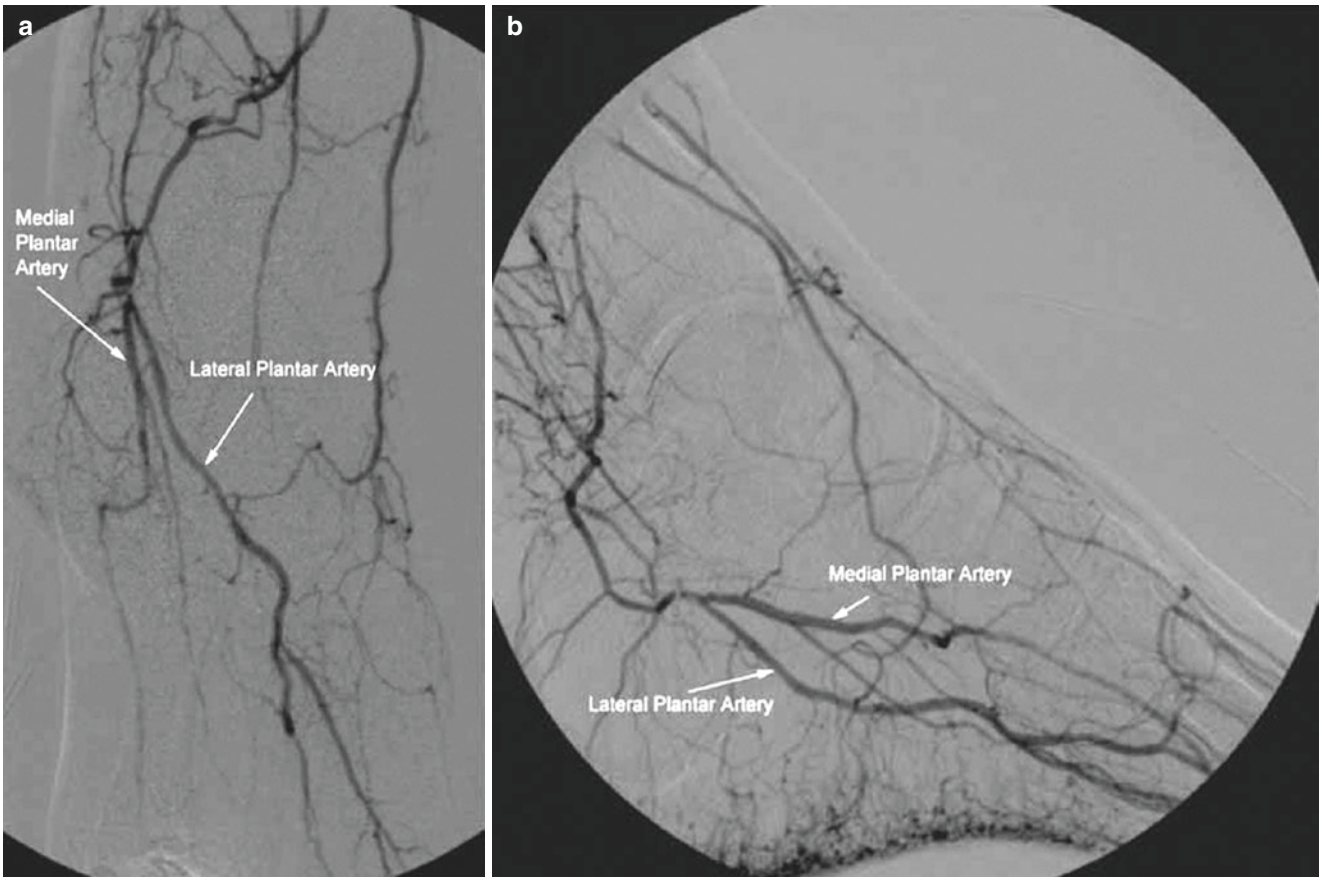

Fig. 20.11 Preoperative anteroposterior (**a**) and lateral (**b**) arteriogram of the foot of a patient undergoing a plantar artery bypass

bypasses, 30-day mortality was 1% and early graft failure, within 30 days, occurred in 11%. In this group, secondary graft patency was 70% at 1 year and 50% at 5 years. Limb salvage was nearly 70% at 5 years [102]. These results are encouraging regarding limb salvage in a group of patients that are all too often advised that limb amputation is the only option by physicians who do not consider extreme distal bypass as a treatment option (Fig. 20.12).

Young patients with juvenile onset Type 1 diabetes mellitus may develop ischemic foot complications from premature atherosclerosis. In distinction to older patients, atherosclerosis in this group is rapidly progressive and associated with a worse prognosis [3, 96, 103]. Younger patients undergoing revascularization have been found to be at increased risk for perioperative complications, have an increased rate of multiple revascularization procedures, and have more frequent progression to extremity amputation. We reviewed all patients under 40 years of age who underwent infrainguinal revascularization at our institution from 1990 to 2000 [104]. Fifty-one patients undergoing 76 lower extremity revascularizations were identified. Type 1 diabetes mellitus was very prevalent, afflicting over 94% of patients. During the follow-up period, 11.8% of patients required additional ipsilateral revascularization, 31.3% required a contralateral bypass graft and in 23.5% major amputation was ultimately necessary. The success rate for secondary procedures was marginal when compared to the primary procedures. The primary patency rate, secondary patency rate, and limb salvage rates were 66.7%, 62.5%, 77.8%, respectively, at 1 year and 44.4%, 41.7%, and 64.8%, respectively, at 5 years. Long-term survival was 75% at 5 years. The results are inferior to those of our older patients where graft patency and limb salvage approach were 80% and 90% at 5 years. The worse outcomes may be due to a more aggressive and rapidly progressive form of atherosclerosis or may be a consequence of the relatively high incidence of dialysis-dependent renal failure in these patients. Like patients on chronic hemodialysis, the observed results are inferior to more "typical" patients and attempts to salvage failed reconstructions were rarely successful. These facts must be discussed frankly with the patient prior to initiating therapy, and treatment should be individualized based on the clinical situation with the realization that, for some patients, amputation may be the best first treatment.

Chronic kidney disease is a complication of long-standing diabetes. As a result, vascular surgeons are often confronted with revascularization decisions in patients with diabetes and ESRD. In the past, patients on hemodialysis have been

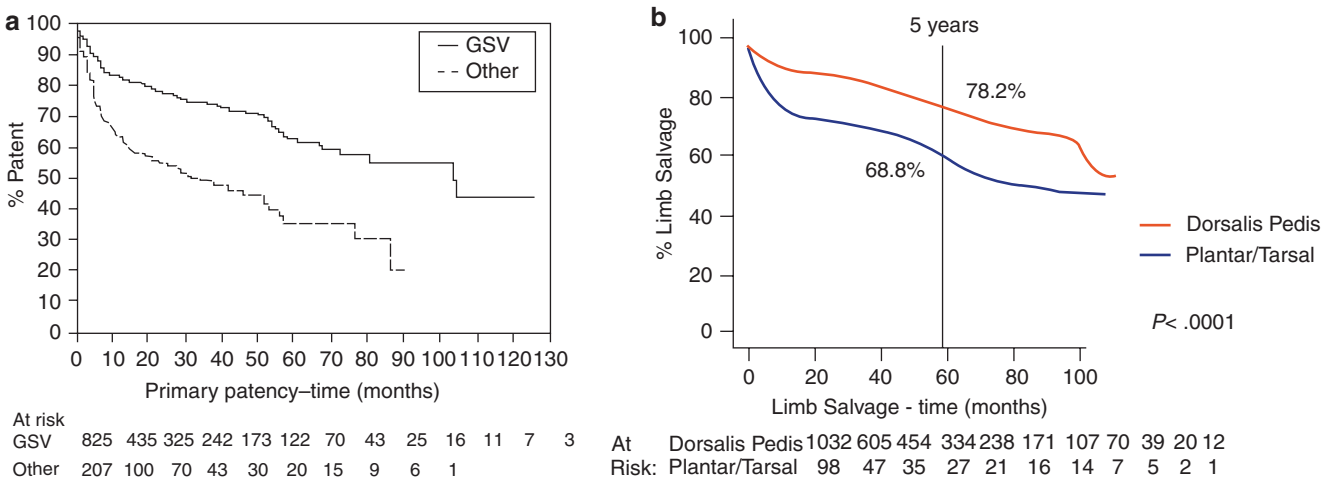

Fig. 20.12 Primary patency (**a**) and limb salvage (**b**) for patients undergoing bypasses to the dorsalis pedis artery and to the plantar/tarsal arteries

deemed to be at a too high risk for surgical bypass. In 2002, we reported our experience with 146 ESRD patients undergoing lower extremity bypass in 177 limbs [105]. Notably, 92% of the study population had comorbid diabetes mellitus and the cause for hemodialysis was DM in 88%. The 30-day mortality rate was found to be 5%, reflecting acceptable perioperative safety. However, overall survival rates at 1, 3, and 5 years were 60, 18, and 5%. Despite this, the 3-year limb salvage rate was 80% suggesting that patients died from causes unrelated to the status of the bypass. Multivariable analysis identified age and number of years on dialysis as predictive of worse outcomes. Subsequently, a meta-analysis of infrainguinal bypass in more than 1000 ESRD patients was performed [106]. The perioperative mortality rate was found to be 8.8%. The estimated 5-year primary patency rate was 50.4%, secondary patency 50.8%, limb salvage 66.6%, and overall survival 27.5%. In patients with ESRD, bypass can result in limb salvage, but limited overall survival is to be expected.

Recommendations

For diabetic patients with chronic limb-threatening ischemia, we believe the WIfI system should be used to assign an initial stage of disease [9]. If limb salvage is attempted, then wound care, infection control, and revascularization are all essential. A diagnostic arteriogram will allow planning for endovascular or surgical intervention. An individualized approach should be used to choose whether to initially pursue endovascular or open surgical treatment. In general, surgical bypass is preferred in patients with good quality great saphenous vein conduit and life expectancy ≥2 years. Bypass is also favored for advanced WIfI stage, multilevel, or long-segment occlusive disease. Endovascular therapy is preferable in patients with high surgical risk and limited life expectancy. Endovascular techniques are also more appropriate when high-quality vein conduit is unavailable and when the occlusion or stenosis is at a single level [93].

Despite the fact that the prevalence of diabetes has increased markedly in recent years, improvements in revascularization techniques and increased utilization of preventative care have resulted in a dramatic decrease in the rate of lower extremity amputations [107]. In order to maintain this trend, vascular disease specialists must be proficient in all forms of revascularization. The breadth of endovascular and surgical options for limb salvage in the diabetic patient has expanded to the point where treatment plans are highly individualized and combinations of techniques are common.

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Soft Tissue Reconstructive Options for the Ulcerated or Gangrenous Diabetic Foot

21

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Abstract

The complex biomechanics of the foot and ankle allow for a highly efficient and coordinated functional unit capable of nearly 10,000 steps a day. However, changes in sensation, motor function, skeletal stability, blood supply, and immune status render the foot and ankle susceptible to breakdown. Inability to salvage the injured foot traditionally has led to major amputation, carrying with it dramatic morbid sequelae and a lifetime dependence on prosthetic devices. Worldwide, a limb is lost to diabetes nearly every 30 s [Young, Lancet. 366(9498):1687, 2005]. Consequently, the relative 5-year mortality rate after limb amputation is greater than 50%, a startling figure when compared to mortality rates of lung cancer (86%), colon cancer (39%), and breast cancer (23%) [Armstrong et al., Int Wound J. 4(4):286–287, 2007].

Because the foot and ankle is such a complex body part, salvage often demands a multidisciplinary team approach. This team ideally should consist of a vascular surgeon skilled in endovascular and distal bypass techniques, a foot and ankle surgeon skilled in internal and external bone stabilization techniques, a soft tissue surgeon familiar with modern wound healing as well as soft tissue reconstructive techniques, an infectious disease specialist to manage antibiotic therapy, and an endocrinologist to help manage the glucose levels. Surgical goals include transforming the chronic wound into an acute healing wound with healthy granulation tissue, neo-epithelialization, and wrinkled skin edges. This may include ensuring a good local blood supply, debriding the wound to a clean base, correcting any biomechanical abnormality, and nurturing the wound until it shows signs of healing. The subsequent reconstruction can then usually be accomplished by simple techniques, 90% of the time and complex flap reconstruction in 10% of cases. This chapter focuses on the critical aspects of limb salvage including evaluation, diagnosis, and treatment with a focus on flap-based reconstructions.

Introduction

The complex biomechanics of the foot and ankle allow for a highly efficient and coordinated functional unit capable of nearly 10,000 steps a day. However, changes in sensation, motor function, skeletal stability, blood supply, and immune status render the foot and ankle susceptible to breakdown. Inability to salvage the injured foot traditionally has led to major amputation, carrying with it dramatic morbid sequelae and a lifetime dependence on prosthetic devices or wheelchair. Worldwide, a limb is lost to diabetes nearly every 20 s [1]. Consequently, the relative 5-year mortality rate after limb amputation is 30–80%, a startling figure when compared to mortality rates of lung cancer (86%), colon cancer (39%), and breast cancer (23%) [2, 3].

Because the foot and ankle is such a complex body part, salvage requires a multi-team approach. This team ideally should consist of a vascular surgeon skilled in endovascular and distal bypass techniques, a foot and ankle surgeon skilled in internal and external (Ilizarov) bone stabilization techniques, a soft tissue surgeon familiar with modern wound healing as well as soft tissue reconstructive techniques, an infectious disease specialist to manage antibiotic therapy, an endocrinologist

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to help tightly manage the glucose levels, a rheumatologist to manage any autoimmune component of the ulcer, a hematologist to manage any coagulopathy, and a primary care physician to manage the patient medically throughout the course of their disease. Surgical goals include transforming the chronic wound into an acute healing wound with healthy granulation tissue, neo-epithelialization, and wrinkled skin edges. Wrinkled skin edges denote a decrease in regional edema following the inflammatory phase of a local infection or acute wound, and wrinkling can be seen in contradistinction to the shiny, tense appearance of the skin during the acute phase. Transforming the chronic wound may also include ensuring a good local blood supply, debriding the wound to a clean base, correcting any biomechanical abnormality, and nurturing the wound until it shows signs of healing. The subsequent reconstruction can then usually be accomplished by simple techniques 90% of the time and complex flap reconstruction in 10% of cases. This chapter will focus on the critical aspects of limb salvage including evaluation, diagnosis, and treatment with a focus on flap-based reconstructions.

Establishing a Diagnosis

History

A thorough patient history is taken which should include the origin (usually traumatic) and age of the wound. Though low-energy, the trauma is usually related to biomechanical abnormalities causing excessive local pressure during gait, changes in shoe wear, penetrating trauma or burn (hot sand or water bath). The patient's tetanus immunization status is obtained and the patient is inoculated if revaccination is indicated. It is important to ask what previous topical therapy was applied to the wound because certain topical agents can contribute to the wound's chronicity [4] (e.g., caustic agents such as hydrogen peroxide, 10% iodine, alcohol). Finally, the nutritional status is assessed: their recent weight gain or loss, the quality of their diet. Their smoking status is documented and a complete list of medications and drug allergies are obtained.

A social history is then obtained to determine the level of physical activity, the level of home help available, and the type of work they are involved in. This can help to assess the patient's ability to comply with the treatment regimen because these wounds can involve up to 6 months of limited activity (i.e., Ilizarov treatment of a Charcot collapse). The diabetic patient's lack of compliance can be as high as 68% [5] is the single biggest reason for postoperative wound complications in excess of 20–55%.

Physical Exam

The wound is then assessed carefully by measuring its size and depth. The approximate area is obtained by multiplying the length of longest axis and by the width of the widest axis perpendicular to it. Depth is measured to assess the approximate area. More accurate measurement is now possible through handheld devices such Aranz Silouhette laser camera (Christchurch, New Zealand) [6]. The exposed layers of tissue are documented: epidermis, dermis, subdermal fat, fascia, muscle tendon, joint capsule, joint, and/or bone. A metallic probe is used to assist in the evaluation of the depth of the wound. If the probe touches bone, there is an 85% chance that osteomyelitis [7] is present. If tendon is involved, the infection is very likely to have tracked proximally or distally. One should check for bogginess proximally and distally along the potentially involved tendon sheaths. If the suspicion is strong that a distal infection has spread proximally, the proximal areas where the tendon sheaths are readily accessible should be aspirated (i.e., extensor retinaculum, tarsal tunnel). The wound is then photographed.

If cellulitis is present, the border of the erythema is delineated with indelible ink, allowing the spread or retreat of the erythema to be continuously assessed and monitored. After debridement, deep cultures of the wound (deep tissue biopsy and not culture swabs) are obtained and broad spectrum antibiotics are started. If after 4–6 h, the cellulitis has extended beyond the inked boundary, either the antibiotics are inadequate and/or the wound has been inadequately debrided (Fig. 21.1). It is important not to confuse cellulitis with *dependent rubor* seen in patients with chronic ischemia or vascular insufficiency. If the erythema disappears when the affected leg is elevated above the level of the heart, then the erythema is due to dependent rubor. With dependent rubor, inflammation is usually absent and the skin should have visible wrinkling. If the erythema persists despite elevation, the wound has surrounding cellulitis and needs antibiotic treatment ± debridement. Dependant rubor can also be often seen at a fresh operative site and should not be confused with postoperative cellulitis. Again, rapid resolution of the erythema with elevation and presence of wrinkled skin at the incision edge indicate dependant rubor rather than cellulitis.

The blood flow to the area is then evaluated by palpation and/or handheld Doppler [8]. The presence of palpable both anterior and posterior tibial pulses suggests adequate blood flow. If one of the pulses is absent, then the pulses should be evaluated with a Doppler. If the quality of flow is questionable, a formal noninvasive arterial Doppler evaluation has to be performed. The posterior tibial, dorsalis pedis and both branches of the peroneal artery should be assessed. Triphasic flow is normal, biphasic flow may be normal, and monophasic flow mandates an angiogram. If the abnormal flow feeds the angiosome where the wound is, an angiogram should be done. The patient should then be referred to a vascular surgeon who *specializes* in distal lower extremity endovascular and bypass revascularizations. In the face of undetermined or inadequate blood flow, debridement should be delayed until

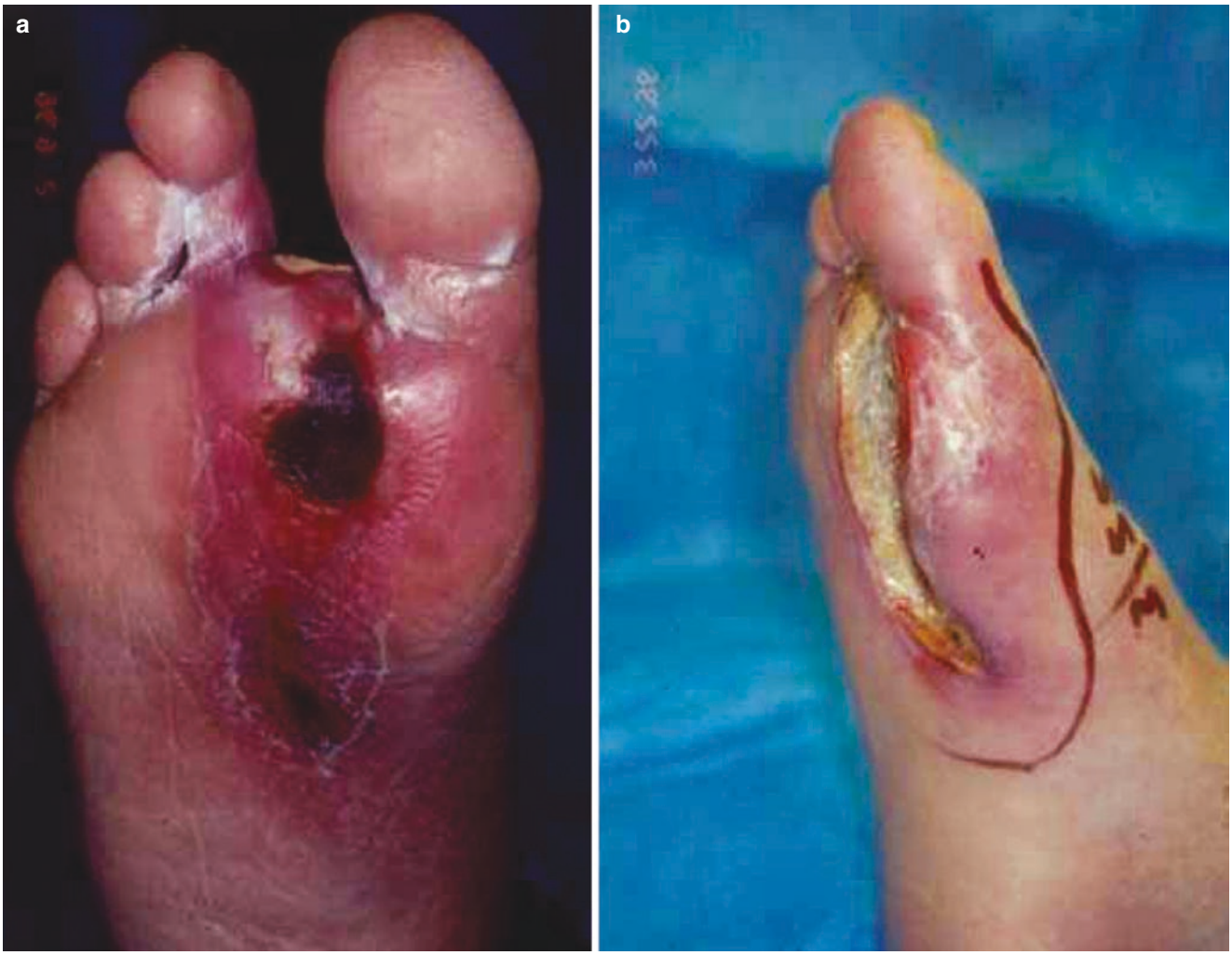

Fig. 21.1 If a foot presents with cellulitis (**a**), the border of the erythema is delineated and dated with indelible ink. If there is necrosis or ulceration, the wound should be debrided. After debridement, deep cultures of the wound are obtained and broad spectrum antibiotics are started. The delineated borders of the initial erythema are then assessed. If, after 4–6 h, the cellulitis has extended beyond the inked boundary, either the antibiotics are inadequate and/or the wound has been inadequately debrided. In this case the redness has receded and therefore the initial therapy is appropriate (**b**)

blood flow status has been assessed and corrected. However immediate debridement is called for regardless of the vascular status when wet gangrene, ascending cellulitis from a necrotic wound, or necrotizing fasciitis is present. The wound can then be kept clean with dressing changes until revascularization. If the wound manifests progressive gangrene, maggots can be applied to locally debride necrotic tissue while the patient awaits revascularization [9, 10]. After successful bypass surgery, it then takes 4–10 days to maximize surrounding tissue oxygen level [11], in comparison to 3–4 weeks following endovascular revascularization.

Sensation must also be assessed. Lack of protective sensation can be established when the patient is unable to feel 10 g of pressure (5.07 Semmes-Weinstein monofilament). A more simple and equally effective assessment is the Ipswich Touch Test where one lightly touches the foot in five places to assess sensitivity to touch. Assessing sensation is critical to help understand the etiology of the ulcer, determine offloading regimens, and prevent recurrent ulceration [12].

Following evaluation of the blood flow and sensation to the extremity, the presence of aberrant or compensatory gait biomechanics should be evaluated. Foreshortening and thickening of the Achilles tendon can cause high focal plantar pressures during gait. This is frequently seen in diabetics as the presence of increased glucose by-products bind to the collagen in tendon and nerves, diminishing the elasticity of these structures. The patient's ability to dorsiflex the neutral foot should be evaluated to confirm the elasticity of the Achilles tendon (Fig. 21.2). If the patient can dorsiflex the foot to greater than neutral (90° to the leg) with the leg straight and bent, then the tendon has sufficient plasticity. If the patient cannot dorsiflex the foot, then the patient has an equinus

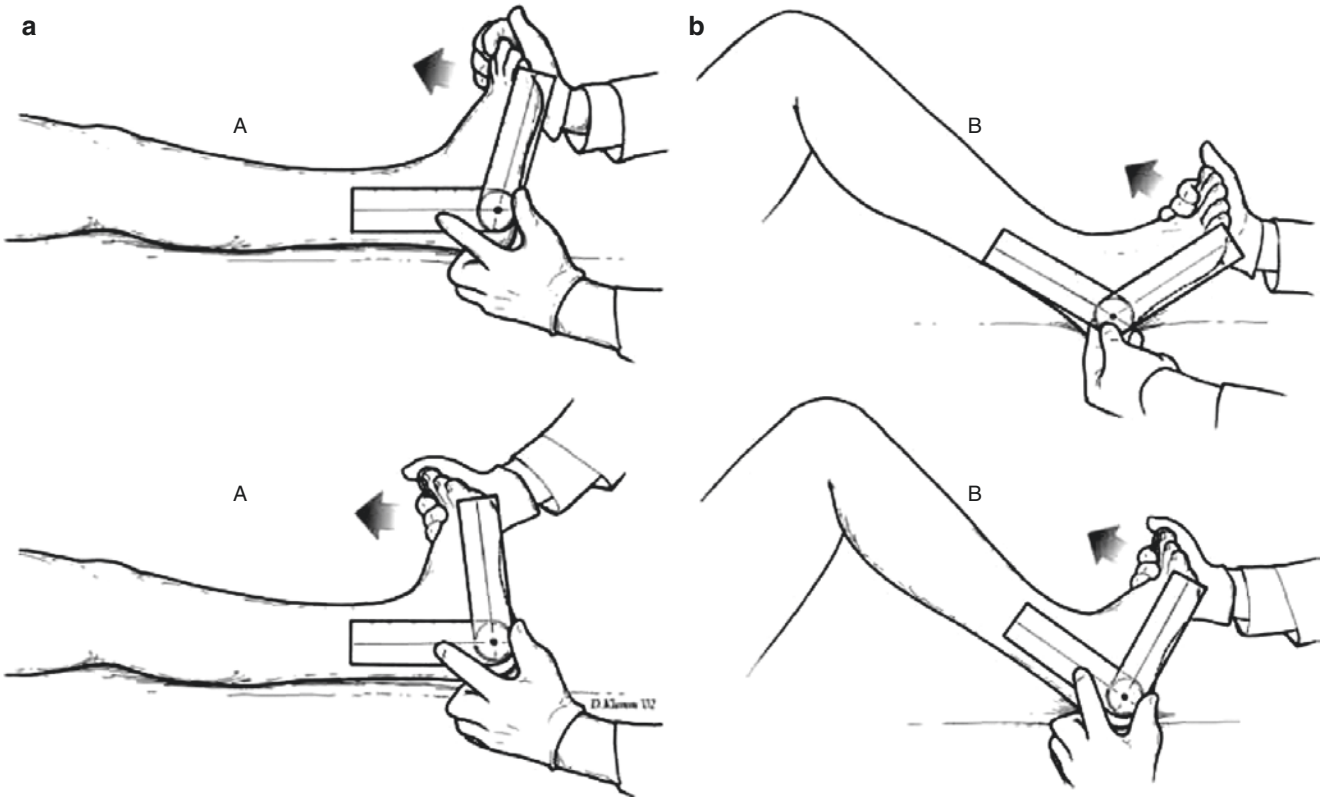

Fig. 21.2 The patient's ability to dorsiflex the supinated foot tests the elasticity of the Achilles tendon. If the patient can dorsiflex the foot greater than neutral with the leg straight (**a**) and bent (**b**), then the tendon has sufficient plasticity. If the foot can only be dorsiflexed when the leg is bent, then the Gastrocnemius portion of the Achilles tendon is tight. If the patient cannot dorsiflex the foot, then the patient has an equinus deformity

deformity. If the foot can only be dorsiflexed when the leg is bent, then the Gastrocnemius portion of the Achilles tendon is tight. If the foot cannot dorsiflex when the leg is straight or bent, then both the Gastrocnemius and Soleus portions of the Achilles tendon are tight. In these circumstances, open or percutaneous release of the Achilles tendon [13] decreases forefoot pressure in the equino-varus foot during gait sufficiently to allow for the rapid healing of plantar forefoot ulcers. In addition, Achilles tendon should be released as a part of any transmetatarsal or midfoot amputation. The release results in a permanent decrease in push-off forces which has been shown to decreasing ulcer recurrence rate from 86% to under 50% at 25 months out from surgery [14, 15]. Unless correction of the underlying biomechanical abnormality is part of the entire treatment plan, debriding and good wound care may prove futile. The recurrence rate for a plantar ulcer due to equino-varus deformity is up to 83% at 2 years and decreases by over half if the tendon has been lengthened.

Testing

Blood work should be obtained following initial evaluation. The immediate blood glucose level and chronic glucose level (hemoglobin A1C) should be assessed. Hemoglobin A1C over 6% indicates poor control of blood glucose levels (7% = avg. plasma glucose level of 170 mg/dL, 8% = 205, 9 = 240, 10% = 275 and 11% = 310). High blood sugar in the face of a low hemoglobin A1C can indicate acute infection. The white blood cell count and differentiation is also very helpful in monitoring systemic infection. The numbers, however, can look deceptively normal in renal failure diabetic patients. A sedimentation rate and PCR can be helpful as a tracking tool during treatment of an infection. The kidney function should be carefully evaluated especially as many of these patients may require an angiogram.

An X-ray is critical to evaluate the underlying bone architecture; however it may be less useful in acute cases as it can take up to 3 weeks for osteomyelitis to appear on X-ray. An MRI is helpful to evaluate possible charcot arthropathy or complex widespread infection. A nuclear scan is usually superfluous if the surgeon plans to evaluate the affected bone or charcot arthropathy during the debridement. However, it can be useful when the extent of osteomyelitis in the suspected bone is unclear or when there is suspicion that other bones may be involved.

Noninvasive arterial studies are useful adjuncts to help assess the quality of blood flow to the foot. Ankle–brachial

indices are inaccurate in diabetics because their arterial walls calcify which then prevents the cuff from compressing the vessel. Because of the calcification, an ABI of <0.9 is deemed abnormal [16]. Because the digital arteries are less likely to calcify, toe pressures higher than 50 mm Hg indicate adequate flow. Pulse volume recordings that contain at least 15 small boxes in height indicate adequate arterial flow volumes. Tissue oxygen levels can be very useful if the laboratory tests them reliably. Levels lower than 20 mm Hg indicate poor healing potential, level between 20 and 40 mm Hg indicate possible healing, and levels higher than 40 mm Hg indicate good healing potential. Skin perfusion pressures have also been used successfully to predict the healing potential of a wound or amputation level [17]. Since no one test is totally accurate, the combination of all the above tests help provide the clinician with a more complete picture of the actual blood flow.

Debridement

The Role of Debridement in Wound Healing

Debriding a wound is defined as removing necrotic tissue, foreign material, and infecting bacteria from wound. Necrotic tissue, foreign material, and bacteria impede the body's attempt to heal by producing or stimulating the production of proteases, collagenases, and elastases that overwhelm the local wound healing process [18]. In this process, the building blocks (chemotactants, growth factors, growth receptors, mitogens, etc.) necessary for normal wound healing are destroyed. This hostile environment is one in which bacteria can proliferate and further inhibit wound healing. Bacteria produce their own wound inhibiting enzymes as well and consume many of the scarce local resources (oxygen and nutrition) that are necessary for wound healing. 99% of the bacteria in chronic wounds reside within a glycocalyx (biofilm) that protects them from destruction by antibiotics and/or WBC and promotes an inflammatory response [19]. The importance of debridement was re-emphasized when Steed reviewed the data of platelet-derived growth factor's effect on the healing of chronic diabetic wounds [20] and observed that wounds healed far more successfully when the wound debridement was performed weekly rather than more sporadically.

When debriding, use of atraumatic surgical techniques to avoid damaging the healthy tissue left behind is mandatory. Healthy tissue should be protected as it is the source of growth factors, nutrients, and building blocks required for subsequent healing. To leave a maximal amount of viable tissue behind, avoid traumatizing techniques such as crushing the skin edges with forceps or clamps, burning tissue with electrocautery, or tying off large clumps of tissue with sutures [21]. Chronic wounds have senescent cells at the edge of the wound that prevent healing [22]. Removal of 3–4 mm of the wound edge in these cases is as important as debriding the wound base in regard to healing capacity.

The principal debriding technique consists of removing the grossly contaminated or ischemic tissue en masse. Surgical tools include a scalpel blade, mayo scissors, curettes, and rongeurs as well as power tools including a sagittal saw and a power burr. However, when debridement approaches viable tissue, the technique is slice thin sheet of tissue after thin sheet of tissue until only normal tissue remains (Fig. 21.3). It is very helpful to use tissue color as a guide; at the end of a successful debridement, only three colors should remain in the wound base: red, yellow, and white. In a similar sense, it is helpful to paint the wound surface with methylene blue dye prior to debridement. The disappearance of all the blue dye ensures that the wound surface has been entirely debrided as it can be easy to miss small areas. These small areas are likely to contain the residual biofilm or bacteria that then repopulate the wound. This is an especially important step not only in definitive debridement but also prior to obtaining deep tissue cultures as many chronic wounds will have mixed flora that are not necessarily involved with deeper levels of tissue penetration or infection.

Curettes with sharp edges are very helpful for removing the proteinaceous coagulum that accumulates on top of both fresh and chronic granulation tissue (Fig. 21.4). A curette will not remove the biofilm that spreads deep to the wound base along the blood vessel providing nutrition to the wound base in a process called perivascular cuffing. Deeper debridement or mechanical energy such as ultrasound is necessary to address that. Also valuable to debridement is the hydrosurgical debrider (Versa-Jet©, Smith & Nephew, Hull, U.K.) that uses a high power water jet (up to 15,000 p.s.i.) to debride tissue. The Venturi effect caused by this high pressure water jet stream sucks the underlying tissue into the stream of water and separates it from the underlying tissue (Fig. 21.5). The debrider works rapidly to take thin slice after thin slice of tissue with minimal surrounding tissue trauma. The chief advantage of the Versa-jet is that it allows for a very accurate control of the depth of cut and hence minimizes the risk of accidentally removing viable tissue.

Debridement should be performed as often as necessary until the post debridement cultures are clean and wound base healthy and ready for reconstruction. The use of biosurgery with maggots is an extremely effective alternative to debriding a wound when the patient cannot tolerate surgery, debriding dressings (wet to dry) or biosurgical agents (Fig. 21.6) [4, 5]. Maggots are the larvae of the *Phoenicia sericata* (green blow fly) and are irradiated so that they cannot metamorphose into the pupae phase. Thirty maggots consume 1 g tissue/day and consume only necrotic tissue and bacteria, leaving any viable tissue intact. Maggots are painless and are very effective against antibiotic-resistant

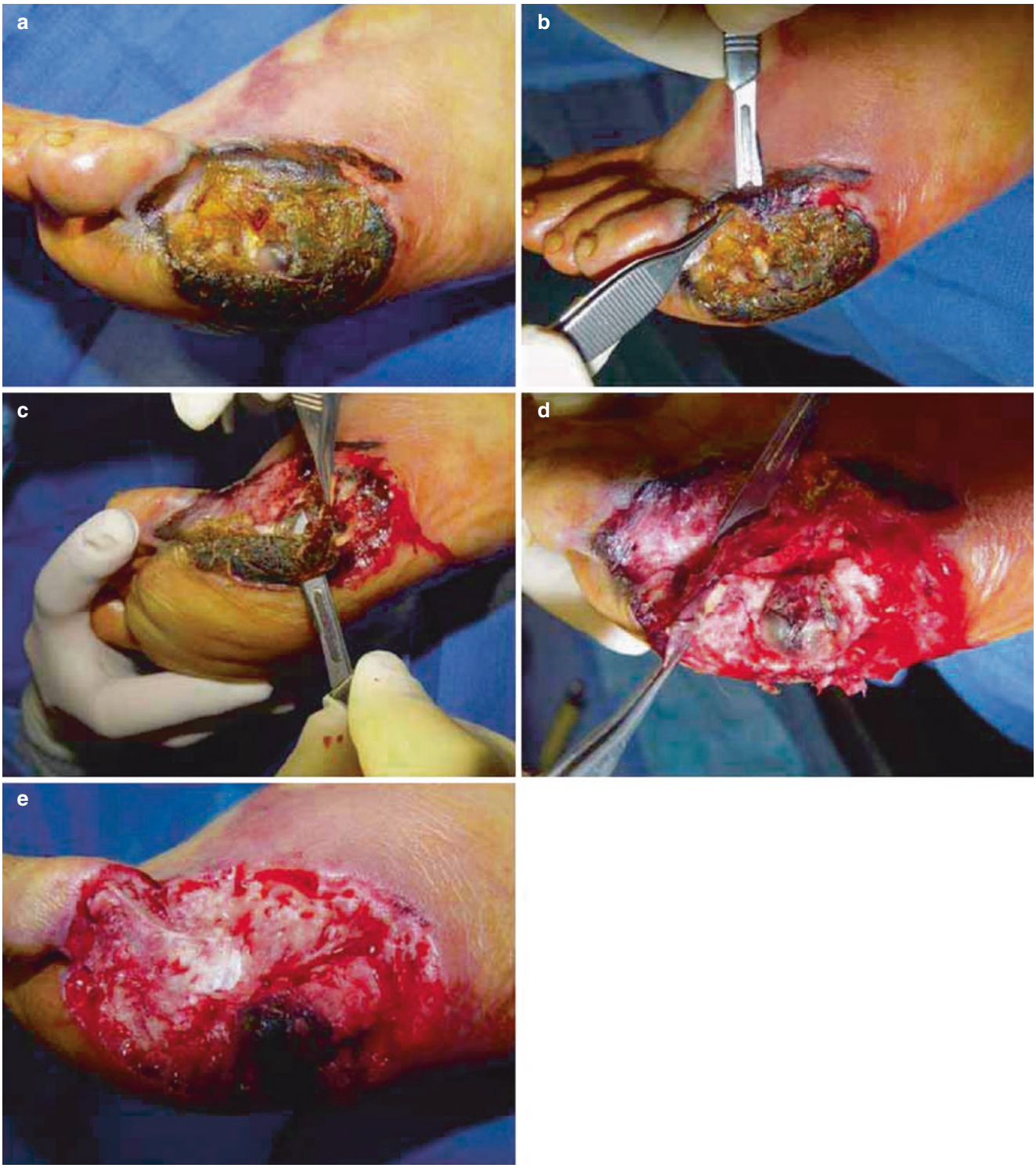

Fig. 21.3 (**a**–**e**) The debridement technique that minimizes the risk of taking normal tissue is to take thin slices after thin slices of necrotic tissue until only normal tissue remains. Note that the remaining colors of the wound are red, yellow, and white. Only the bone needs to be debrided. The technique consists of grasping the tissue to be removed with the pickup and use a #10 or a #20 blade to slice off thin layer after thin layer. Change surgical blades frequently, as they dull quickly

organisms. Maggots are the only agents that destroy all antibiotic-resistant bacteria including *MRSA* or *VRE*. They are applied on the wound and covered with a semipermeable dressing. They are changed every 2 days. However, to use them, one must first obtain the cooperation of both the patient and hospital staff.

In between debridements for outpatient wounds, topical agents can help reduce the bacterial load: ¼ strength acetic

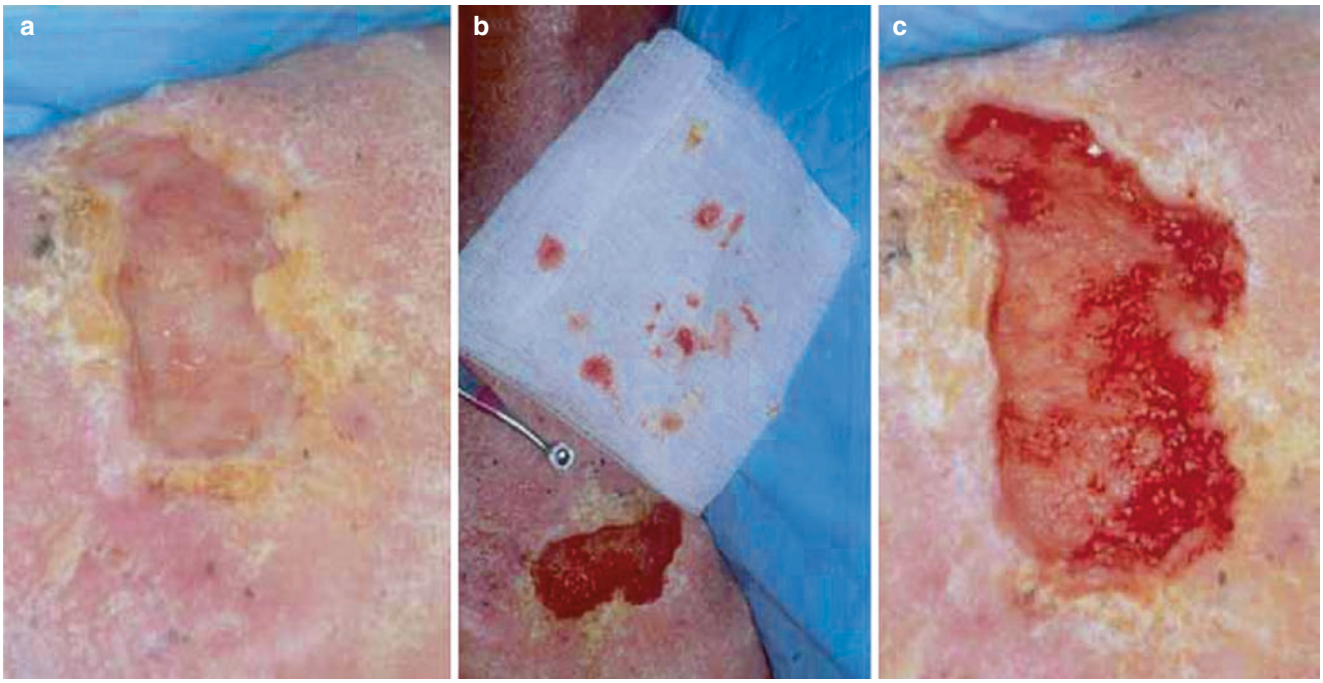

Fig. 21.4 Curettes with sharp edges are very helpful for removing the proteinaceous coagulum and biofilm (**a**) that accumulates on top of both fresh and chronic granulation tissue. A curette is the ideal tool to remove coagulum and superficial biofilm. (**b**). Since the coagulum contains a high concentration of metallo-proteases and biofilm, its removal diminishes the factors that have allowed the inflammatory phase to persist. (**c**) It is important to note that a curette does not remove the biofilm that is present below the surface of the wound which requires other methods to address it

acid, mafenide, silver-sulfadiazine, and silver-containing hydrocolloids can be effective for all wounds. For draining wounds, iodosorb and absorptive alginates work well. Bactroban© is useful for MRSA, Acetic acid or gentamycin ointment for Pseudomonas infections, bacitracin for minimally infected wounds. To address biofilm, a variation of iodosorb, silver, and lactoferrin all can be effective. For inpatient wounds post surgical debridement, negative pressure wound therapy (NPWT/NPWTi) with instillation has proved to be effective in decreasing the length of stay and shortening the time to final surgery by reducing the bacterial count and promoting granulation.

What to Debride

Remove nonviable skin as soon as possible unless revascularization is pending. If the border between live and dead tissue is clearly demarcated, excise the skin just beyond that border. Clotted venules at the skin edge indicate that the local microcirculation has been completely interrupted and that further excision is necessary (Fig. 21.7). Only when there is normal arterial and venous bleeding at the edge of the wound can one be satisfied that the cutaneous debridement has been adequate. Healthy fat has a shiny yellow color and is soft and resilient while dead fat has a gray pallor to it, is hard, and is not pliable. Debride fat until soft, yellow, normal-looking fat appears. Local wound induration and fibrosis can help guide debridement by direct tactile feedback. Healthy fascia has a hard, white, glistening appearance. When dead, it looks dull, soft, and stringy and is in the process of liquefying. Debride all necrotic fascia until solid, normal-looking bleeding fascia or healthy underlying fascia appears. Debriding can be simplified to serially removing tissue until one gets to normal color tissue where one sees only healthy red, white, and yellow in the wound.

Infected necrotic tendon looks dull, soft, and partially liquefied. To ensure that any hidden necrotic tendon is also removed, make a proximal and distal incision along the path of the exposed tendon (Fig. 21.8). Alternatively, pressure can be placed proximally or distally to the wound along the course of the tendon, and the tendon sheath "milked" to determine the presence of tracking infection. When the extensor tendons on the dorsum of the foot become exposed, it is hard to preserve them unless they are quickly covered with healthy tissue or with neodermis and then a skin graft. With the larger Achilles or anterior tibial tendon, debride only the portion that is necrotic or infected. Leave the hard, shiny tendon underneath intact. The remaining tendon must be kept moist and clean, as it will granulate.

Healthy muscle has a bright red, shiny, and resilient appearance, and it contracts when grasped with forceps or touched with cautery. In neuropathic patients, the muscle may have a pale, possibly yellowish, color and may appear

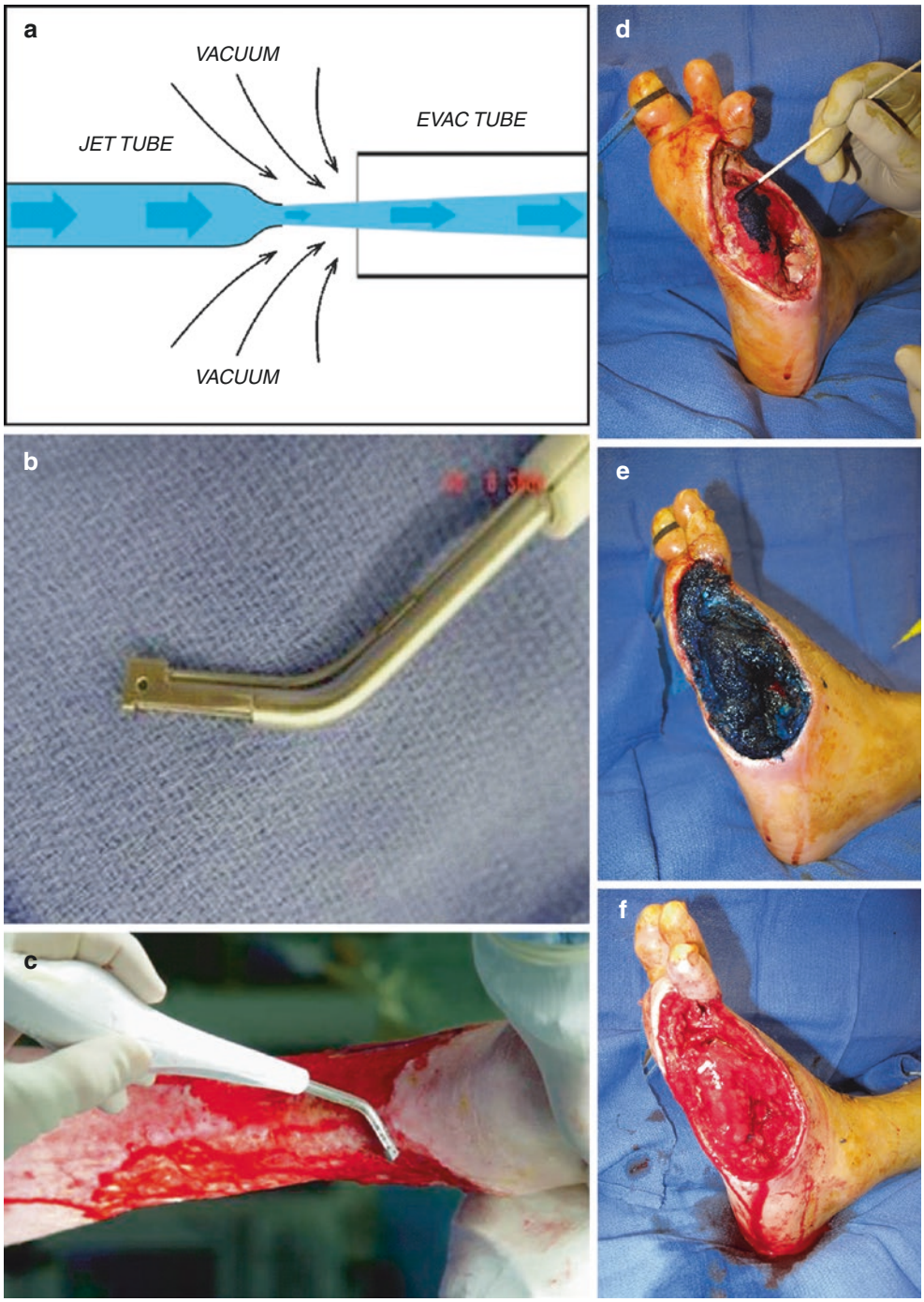

Fig. 21.5 The hydro-surgical debrider (Versa-Jet, Smith & Nephew, Hull, UK) uses a high power water jet (up to 15,000 psi) to debride tissue. The Venturi effect caused by this high pressure water jet stream sucks the underlying tissue into the stream of water and separates it from the underlying tissue (**a**). The debrider (**b**) should be moved back and forth rapidly as it takes thin slice after thin slice of tissue with minimal surrounding tissue trauma (**c**). In order to ensure that the entire wound surface has been addressed, it is useful to paint the surface of the wound with methylene blue (**d**, **e**) and debride until all the blue is gone (**f**). Of course, the base of the wound should only have normal colors at its base including yellow, red, and white

nonviable. It will have some tone, however, and will bleed when cut. Frankly dead muscle will be swollen, dull, and grainy when palpated, and it falls apart when pinched. If the muscle's viability is questionable, err on the side of caution and remove only what is not bleeding and appears dead. Subsequently, serially debride the wound until only viable muscle remains.

The key to debriding bone is to remove only what is dead and infected and leave hard bleeding bone behind. In the larger bones, use a cutting burr to remove thin layer by thin layer of bone until punctate bleeding (paprika sign) appears (Fig. 21.9). Obtain cultures of the bone remaining after debridement as well as of the debrided osteomyelitic bone to better judge the effectiveness of the debridement. If the post debridement bone culture is clean after all infected bone has been removed, just 1 week of appropriate antibiotics is necessary post operatively [23]. Only when there is a question that when the bone left behind (e.g., calcaneus or tibia) may

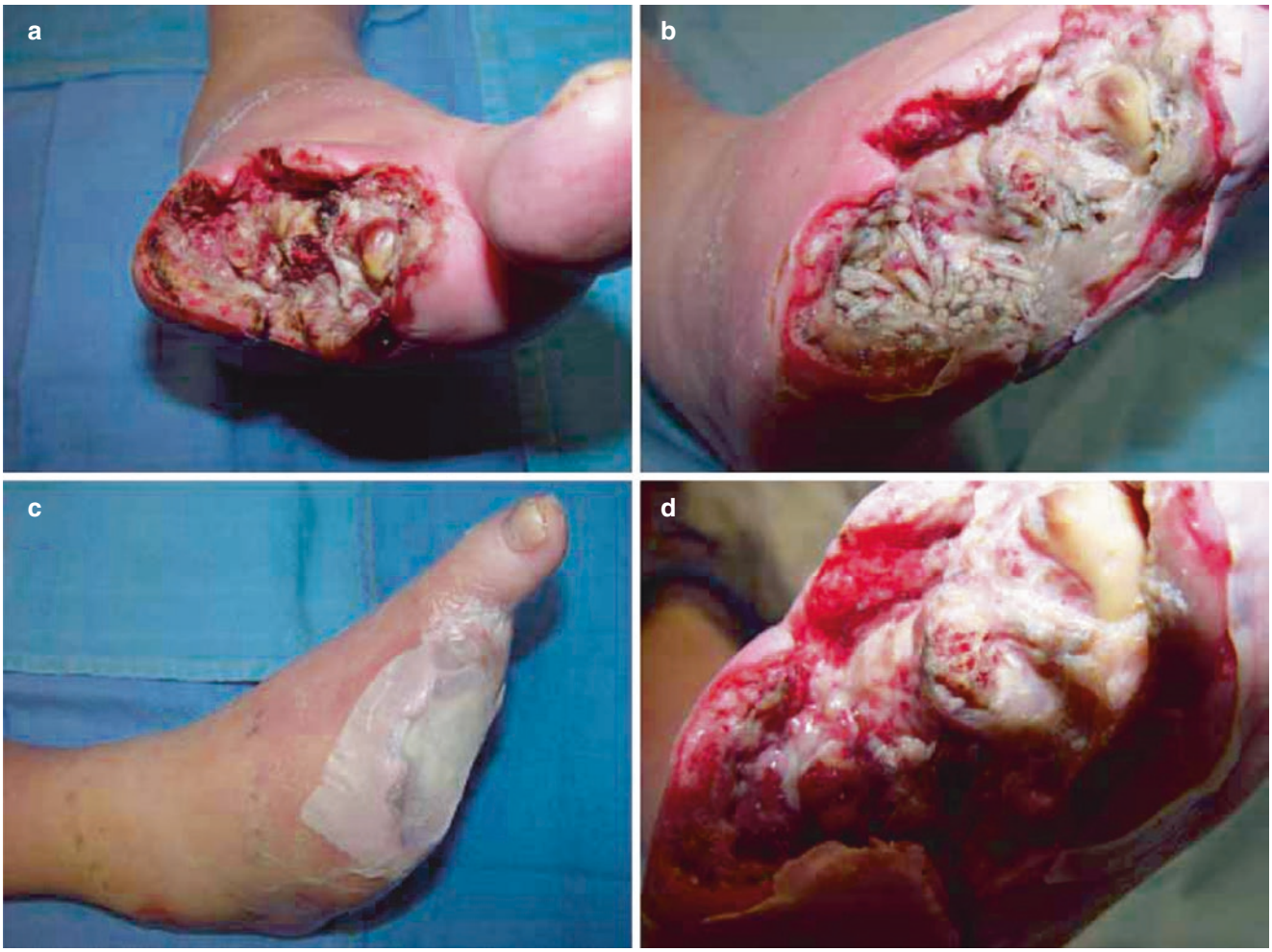

Fig. 21.6 The use of bio-surgery with maggots is an extremely effective alternative to debriding a wound when the patient cannot tolerate surgery, debriding dressing or topical agents. Maggots are the larvae of the *Phoenicia sericata* (green blow fly) and are irradiated so that they cannot metamorphose into the pupae phase. Thirty maggots consume 1 g tissue/day, consuming only necrotic tissue and bacteria and leaving any viable tissue intact. This partial necrotic forefoot (**a**) has maggots placed on it to prepare the wound for closure (**b**). The wound is sealed with a semipermeable membrane so that the maggots cannot escape (**c**). After 2 days of treatment, the wound's edges are clean and have begun to granulate (**d**)

still harbor osteomyelitis, either re-debridement or a longer course of antibiotics is required.

Negative Pressure Wound Therapy (NPWT) or NPWT with Installation

Once the wound is clean and adequately vascularized it can be covered with a NPWT dressing. The NPWT applies negative pressure to a wound via a closed suction mechanism [15, 24]. This speeds up the formation of granulation, decreases bacteria, and reduces tissue edema. The mechanisms by which this occurs are poorly understood. However it is felt that the removal of inhibitory wound healing factors, decrease in edema, increased blood flow, as well as the alteration of the cellular cytoskeleton play a role in sterilizing the wound and stimulating the rapid formation of new tissue (Fig. 21.10). If the sponge is over a potential weight-bearing portion of the foot (i.e., heel), a sponge bridge is attached to the site so that the drainage port is now on a non-weight-bearing portion of the foot. The proximal end of the evacuation tube is then connected via a drainage canister to an adjustable vacuum pump. The subatmospheric pressure can be applied in a constant or intermittent mode with pressures up to 125 mm Hg. The intermittent mode has been found to stimulate the formation of granulation tissue more rapidly and maintain increased blood flow for longer periods of time.

There is substantial evidence that when NPWT with instillation (NPWTi) is added it provides better clinical outcomes than with NPWT alone [25]. NPWTi should not be routinely used to treat simple wounds, but instead utilized

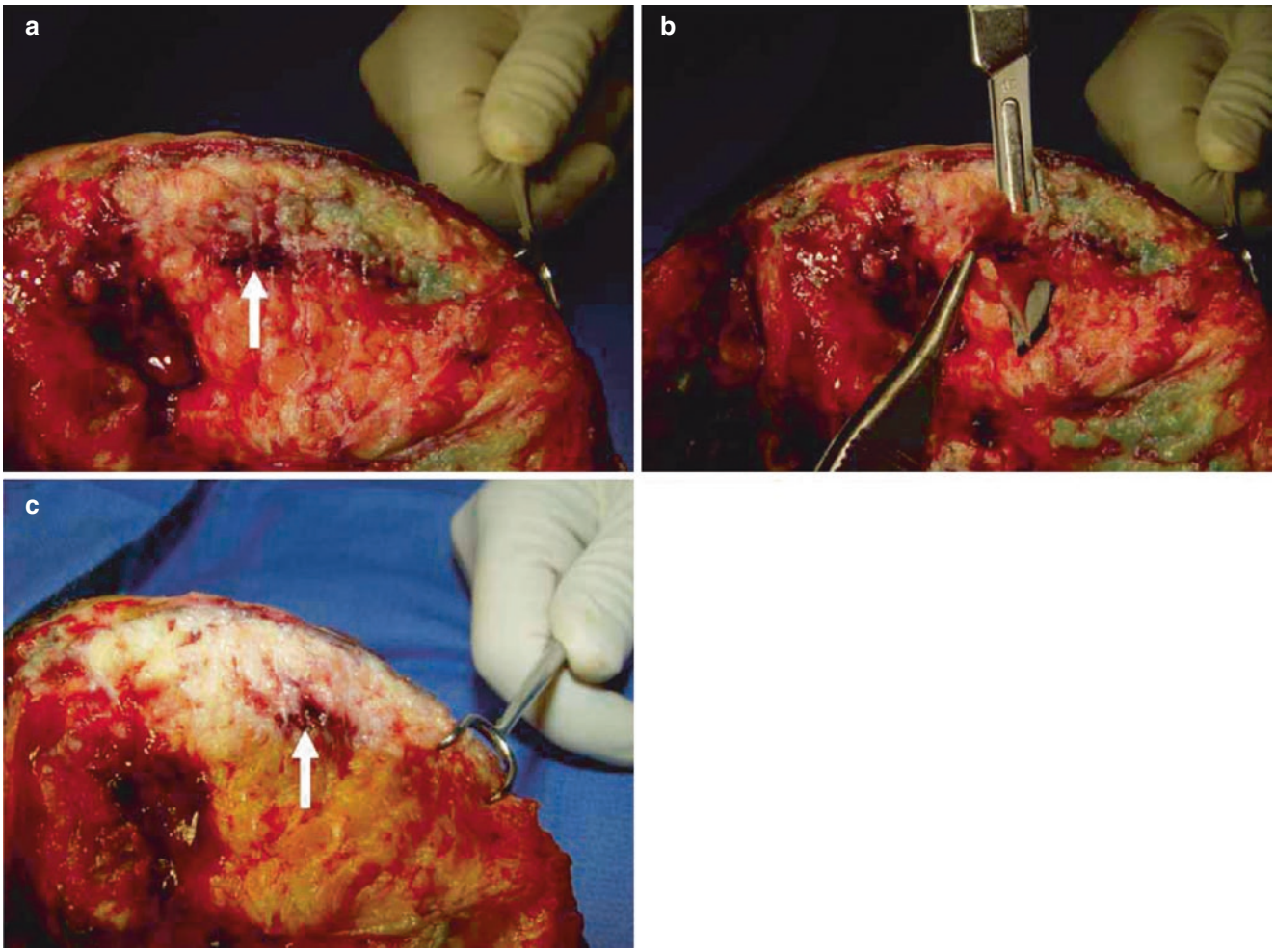

Fig. 21.7 When excising skin, look for bleeding at the normal skin edge. Clotted venules at the skin edge (**a**) indicate that the local microcirculation has been completely interrupted and that further excision is necessary. Thin slice after thin slice of the tissue containing clotted veins (**b**) should be removed until normal tissue appears (**c**). Note that in the final picture there is still a small localized area of clotted tissue that needs to be removed

for patients who are complex hosts or have complex wounds or both. Unlike standard negative pressure wound therapy, NPWTi has been shown to reduce bio-burden and infection in both clinical and basic science studies, and its use as a hospital postsurgical dressing shows a reduction of hospital stay and number of operations. Recommended dwell times of the instillate should be 10 min with a maximum of 20 min and a negative pressure time of 2–4 h at a pressure of −125 mm Hg although larger wounds may need longer times of up to 6 h. Normal saline (0.9%) is the preferred solution for NPWTi except for special situations [26]. For wounds involving hardware, polyhexanide has been shown to be successful in salvaging infected total joints [27]. NPWTi does not replace appropriate wound assessment, fundamental wound care principles (e.g., debridement or offloading), systematic antibiotic therapy, or medical management of cases.

If NPWT or NPWTi is being placed over sensitive structures such as a neurovascular bundle or a tendon, Vaseline mesh (Adaptec©, Johnson & Johnson Gateway, LLC, Piscataway, New Jersey) or silicone mesh (Mepitel©, Mölnlycke Health Care, Göteborg, Sweden) should be placed between the wound and sponge to minimize potential damage to the underlying structure.

The quality and quantity of the granulation tissue is more vascular than that normally produced without the

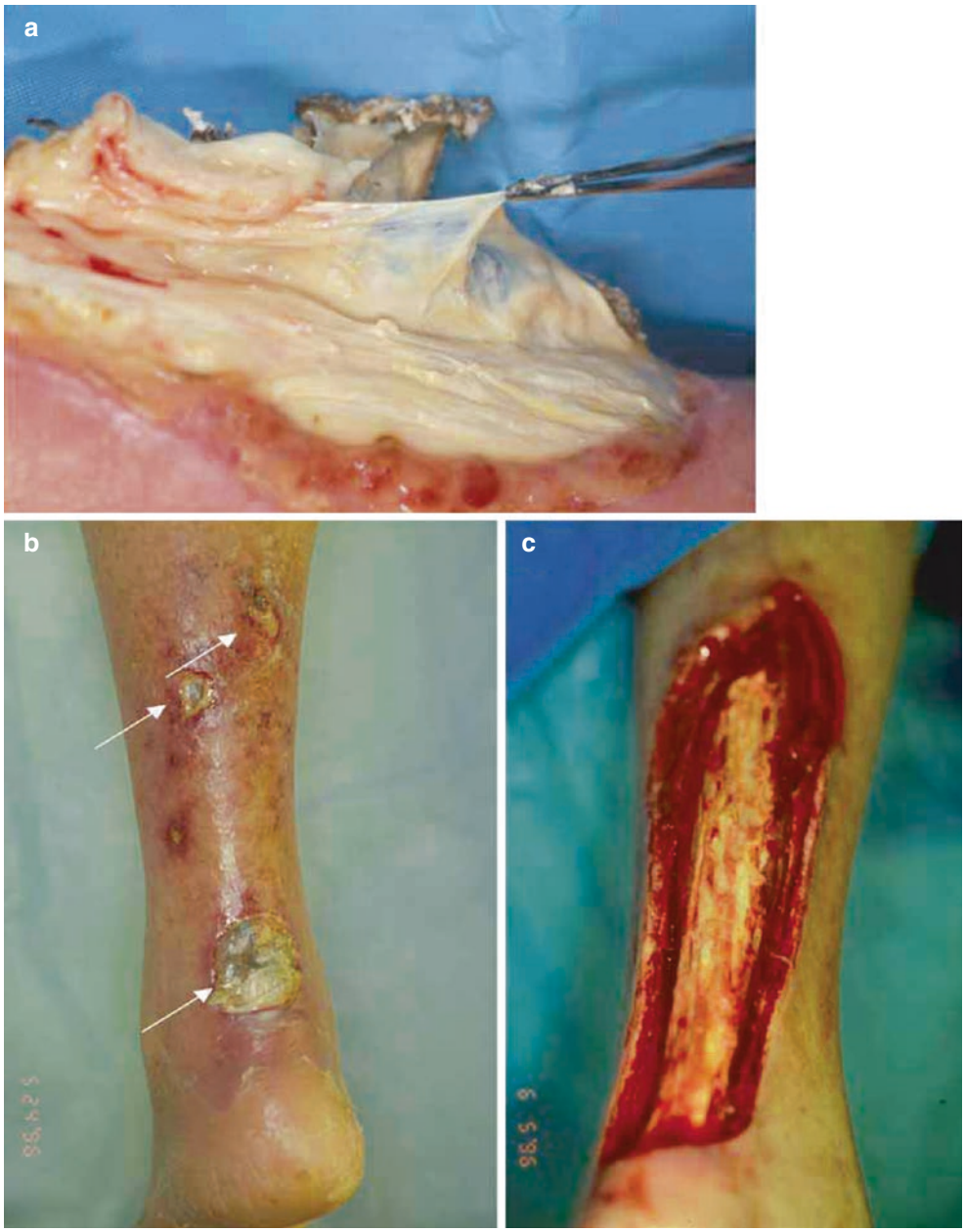

Fig. 21.8 Infected necrotic tendon looks dull, soft, and partially liquefied (**a**). It should be debrided to clean hard normal-looking tendon. For smaller tendons it usually means loss of that tendon. However, for the necrotic Achilles or anterior tibial tendon, much of the tendon can usually be spared. Note that in picture b, the lesion originated at the distal tendon and spread proximally to the mid calf (**b**). To ensure that all necrotic tendon is removed, it is therefore important to explore proximal and distal to the exposed tendon to make sure all necrotic tendon has been removed (**c**)

negative pressure dressings, and small wounds can heal more rapidly [28]. If more involved reconstruction is planned, NPWT or NPWTi gives the surgeon time to electively cover the wound [29] with a microsurgical free flap. In addition, the reconstructive plans are usually simplified because the NPWT or NPWTi shrinks the size of the wound so that most wounds can be closed with a combination of local flaps and skin grafts. The NPWT or NPWTi has limited effectiveness if the surgeon expects it to heal a wound over an exposed fracture or joint. In those cases, the safer option is to cover the exposed joint or fracture with a local, pedicled or free flap.

When Is the Wound Ready to Close?

The wound is ready to close when all the abnormal parameters surrounding the wound have been corrected and all signs of inflammation have disappeared (Fig. 21.11). It can then be allowed to heal by secondary intention, be closed

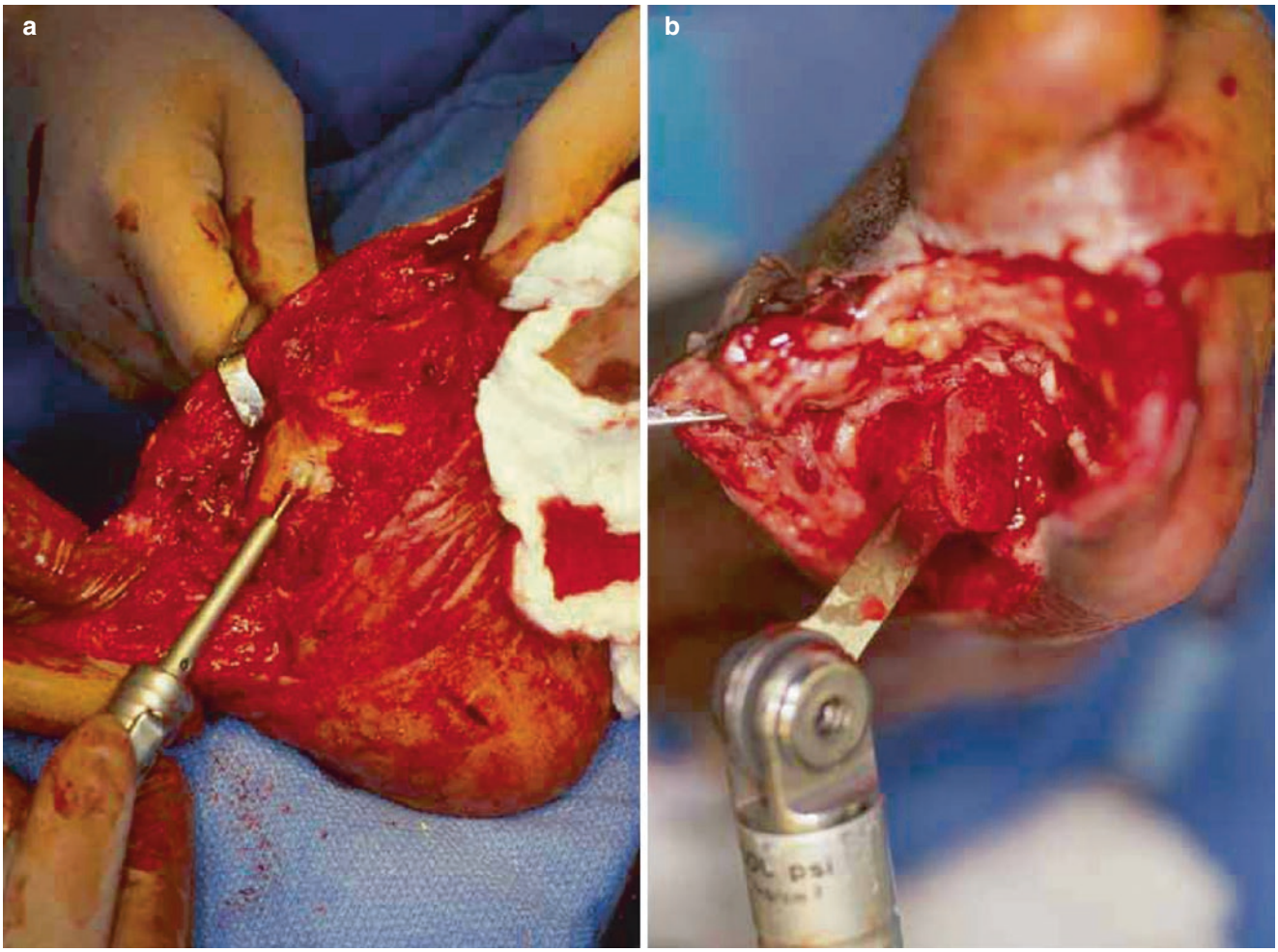

Fig. 21.9 In the larger bones, use a cutting burr to remove thin layer by thin layer of bone until punctate bleeding (paprika sign) appears (**a**). Copious irrigation is necessary to ensure that the heat generated by the burr does not damage the healthy bone The best way to debride the osteomyelitic smaller long bones (phalanx, metacarpals, or metatarsals) is to cut slices of bone serially until normal bleeding bone appears (**b**). When normal bone appears, a culture should be taken and labeled as clean bone so the surgeon and infectious disease specialist can judge whether the proximal bone is free of infection

by delayed primary closure or skin graft or covered with a flap. The wound itself should have no surrounding erythema. Cellulitis should not be confused with dependent rubor due to ischemia or recent local surgery. Wrinkled skin lines at the wound's edge are one of the most reliable signs that inflammation has largely resolved. Induration may be absent in patients who lack normal immunological response (i.e., renal failure, steroid dependence). Pain should have subsided in a wound with resolving inflammation. Decreasing pain, however, is a less reliable indicator than resolving erythema or induration. The latter shows that there is sufficient blood supply and a hospitable environment for the wound to go through the final stages of wound healing. The presence of new epithelium at the wounds edge reflects a healthy wound that is on its way to healing by secondary intention. If quantitative counts are available, then a count of less than 10^5 bacteria per gram of tissue signifies that the wound is ready to successfully be skin grafted [30]. Alternatively, if an allograft or xenograft placed on the wound takes, then the wound bed is sterile enough for a skin graft to take. We have found that in patients who have multiple comorbities and/or are immune-compromised, negative post debridement cultures have higher healing rates [31]. Use of NPWTi between successive debridements and reconstruction has been invaluable in keeping the debrided wound sterile.

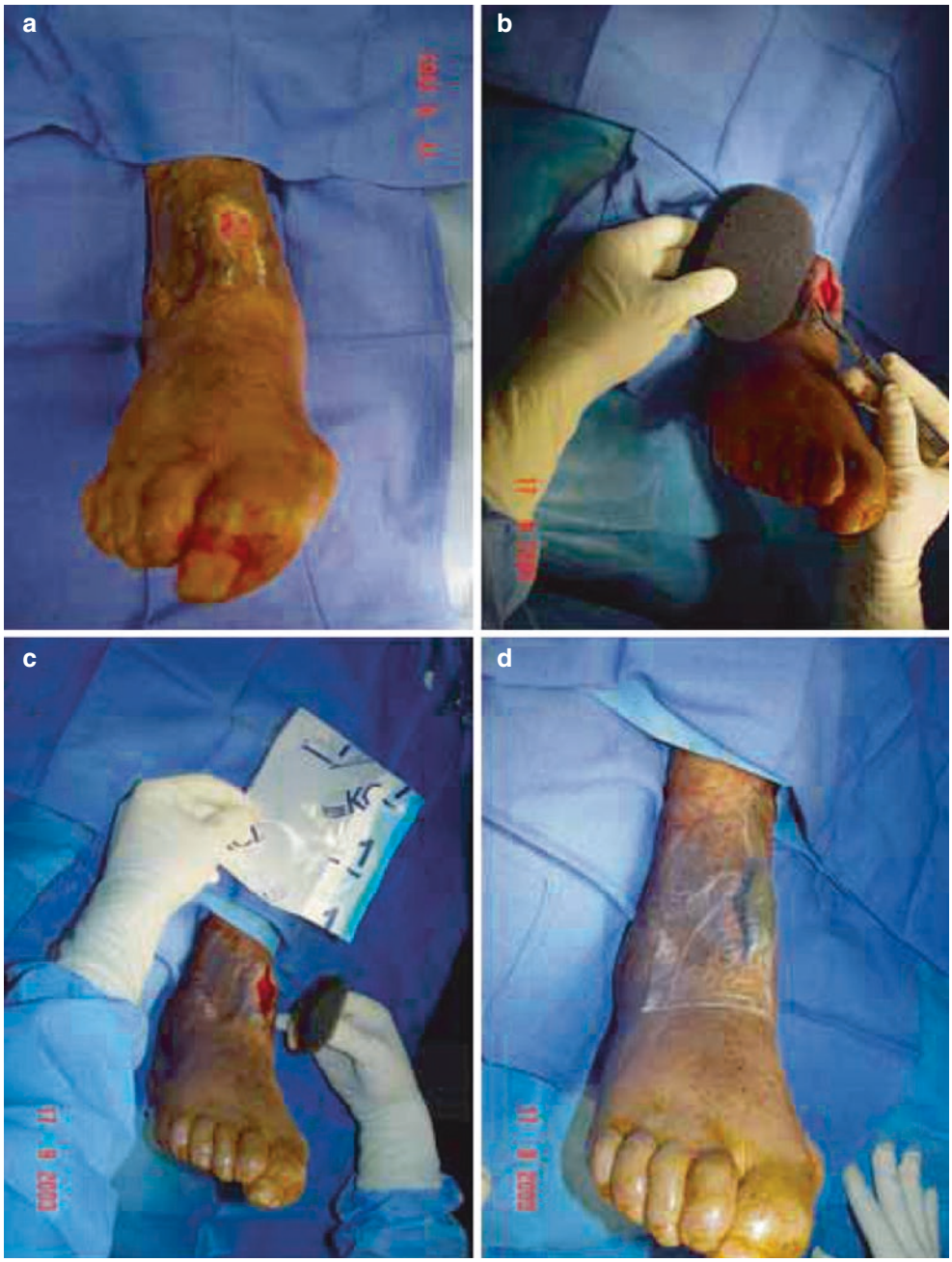

Fig. 21.10 The NPWT system can consist of a polyurethane ether foam sponge or cotton sponge which is placed directly on the wound surface (**a**). A scissor or scalpel blade is used to tailor the shape of the sponge to the contours of the wound (**b**). The wound, with sponge, is then covered with an impermeable adhesive drape that extends 3–5 cm over the adjacent normal skin (**c**, **d**). A small hole is made in the impermeable sheet over the sponge (**e**, **f**). The distal end of the evacuation tube is placed over the fenestration (**g**, **h**). The proximal end of the suction tubing is then connected via a drainage canister to an adjustable vacuum pump. The pump creates subatmospheric pressure that is then applied to the entire wound surface

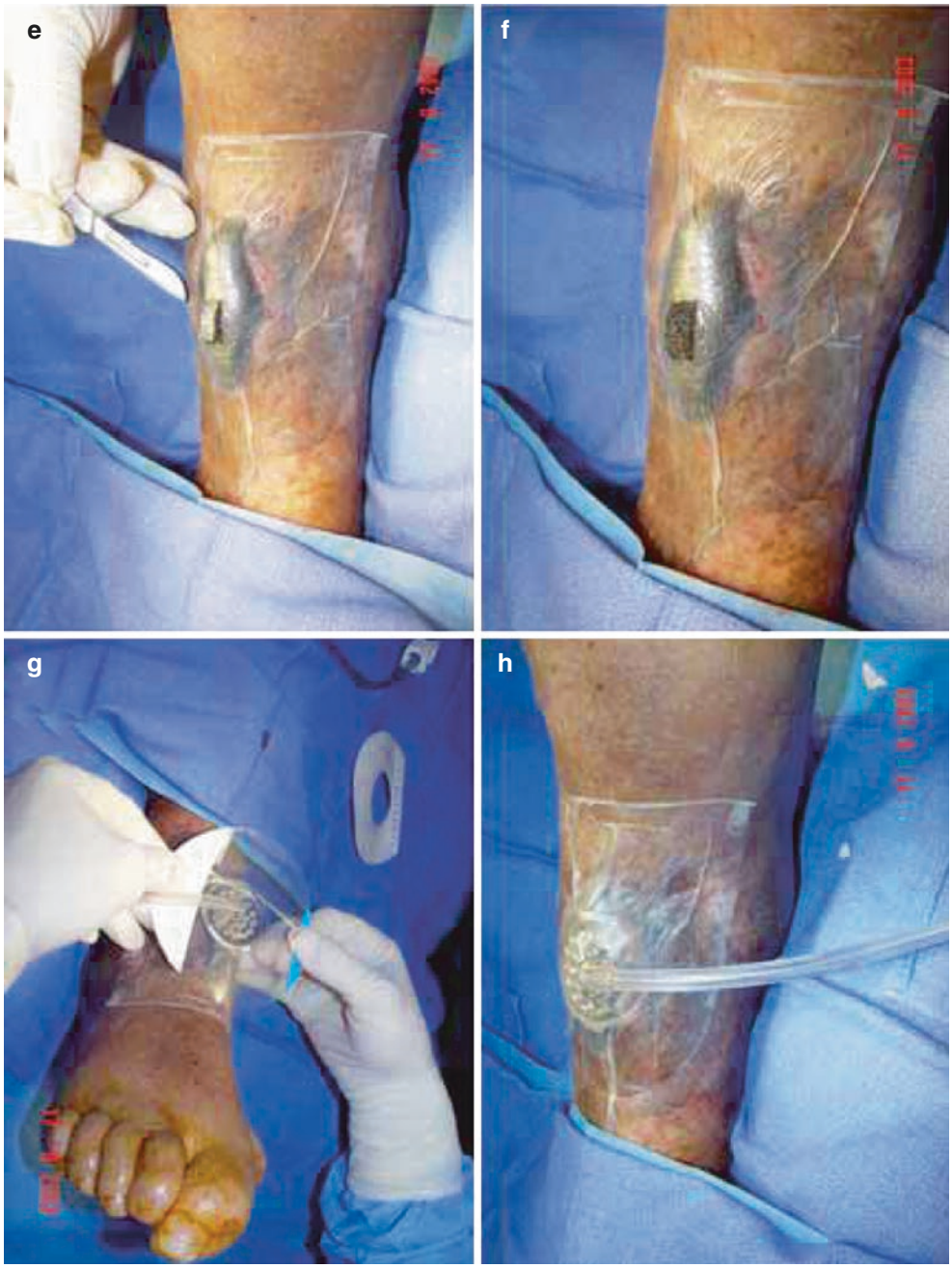

Fig. 10 (continued)

Closure Techniques

Closure techniques include allowing the wound to heal by secondary intention or by closing it with (1) delayed primary closure, (2) skin graft, (3) local flap, (4) pedicled flap, and (5) free flap. If surgical closure is chosen, there should be two setups of instruments in the operating room: one for the debridement and one for closure. This is to avoid contaminating the just debrided wounds with dirty instruments.

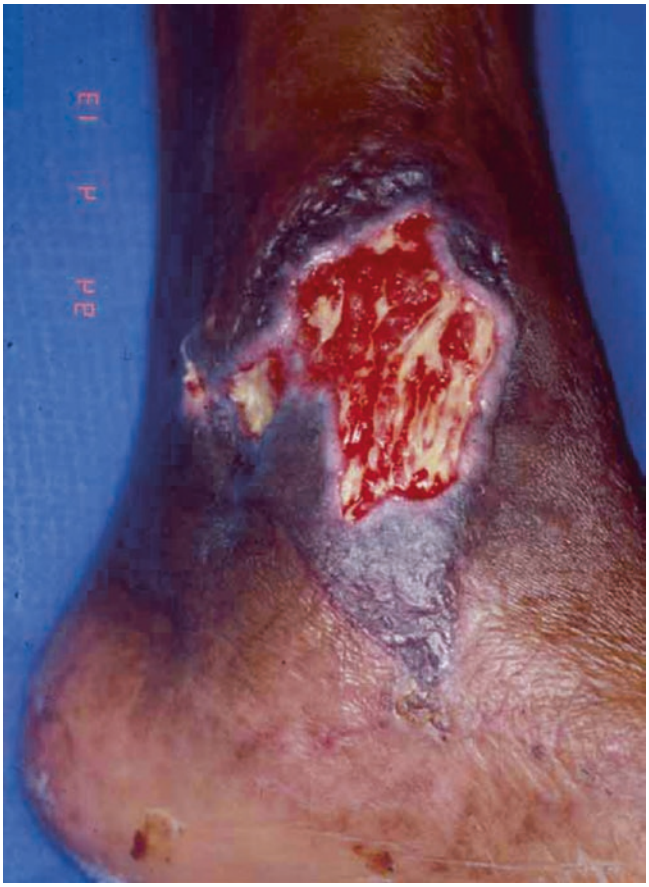

Fig. 21.11 The wound is ready to close when all signs of inflammation have disappeared: erythema, induration and swelling. There should be wrinkled skin lines at the edge of the wound and neo-epithelialization occurring at the border of the wound. The wound can then be allowed to heal by secondary intention, closed by delayed primary closure, skin grafted, or covered with a flap

Promoting Healing by Secondary Intention

A healthy granulating wound should normally decrease in surface area by at least 10–15% per week [32]. The biomechanical abnormality that caused the wound should be addressed. If the wound is on the plantar forefoot and the etiology is an equino-varus deformity from a tight Achilles tendon, and/or a hammer toe, the Achilles tendon should be lengthened and/or the hammer toe corrected. The plantar foot should then be unweighted. If the wound is located near a joint surface (i.e., ankle), the involved joint should be immobilized by a splint or external fixator to prevent shear forces from disrupting the ongoing repair. A moist dressing on the wound allows for more rapid epithelialization of the wound [33]. If the wound fails to respond to the above conservative measures, healing adjuncts should be implemented.

When dealing with wound healing adjuncts, it is important to keep their cost in mind. Xenograft costs approximately $50 per role of 400 cm^2, growth factor $400–$500 per 15 g, and cultured skin derivatives up to 900$ per 25 cm^2 of tissue. Application of the NPWT is approximately $125 per day while hyperbaric oxygen costs in excess of $500 per day. In order to accurately estimate the total cost of a given option, one also has to factor in the cost of visiting nurses, hospital stay, and operative costs. As there is no adequate level one evidence for any of these approaches [34], one should first start with the least expensive clinically appropriate tool and move up the ladder when a given treatment fails to bring about the desired results.

1. *Platelet-Derived Growth Factor*: This gel has been shown effective in diabetic wounds when they are well vascularized, clean, and regularly debrided [35]. Removing the proteinaceous coagulum from the wound surface before applying the growth factor is important because the coagulum contains metallo-proteases that will digest the applied growth factor before the latter can affect the wound. Patients are given scrub brushes or soft toothbrushes and are instructed to scrub the wound surface every time before applying the growth factor.
2. *Temporary Coverage*: Xenograft (pigskin) [36] or allograft (cadaver skin) [37] provides an excellent temporary dressing over clean healthy wounds. They are an excellent temporary dressing that provides a collagen-based scaffolding for new tissue to grow into. If the temporary graft initially "takes," it turns pink indicating that the underlying bed is sterile and well enough vascularized for a split-thickness skin graft to successfully take. In healthy patients, rejection starts at approximately 7–9 days. In the immune-compromised patient, it can take up to a month before the temporary skin graft is rejected.
3. *Cultured Skin Substitutes*: While these are not skin grafts per se, as they do not provide an intact epidermis, they are bioengineered skin equivalents that provide a moist living surface producing an entire array of local growth factors to the underlying wound bed. As opposed to definitive revascularization and ingrowth, they instead

create a scaffolding for native fibroblasts. This scaffolding can then be covered with a layer of epidermis harvested as a skin graft. These products come in several commercial forms: combined dermal-epidermal graft, keratinocyte graft, or a dermal graft. They have been shown to be effective in healing both venous stasis ulcers [38, 39] and diabetic ulcers [40–42]. Collagen matrices have been shown to be effective as a scaffold for cell migration to recreate a live dermis that can then autoepithelialize or be skin grafted [43].

4. *Hyperbaric Oxygen*: Hyperbaric oxygen has been shown to be effective in helping treat radiation-induced injuries, threatened flaps, wound beds of failed skin grafts, and osteomyelitis that has failed antibiotic therapy. Hyperbaric oxygen supplies the body with oxygen at two to three times normal atmospheric pressures. Hyperbaric oxygen saturates existing hemoglobin and dissolves sufficient free oxygen in the blood plasma to increase the concentration of oxygen at the wounds edge. The increase of oxygen at the wound's edge significantly increases the oxygen gradient between the edge and the hypoxic center of the wound bed. The higher the gradient, the stronger the body's wound healing response [44, 45] and more rapid the promotion of angiogenesis, collagen synthesis, and neo-epithelialization. It also has been shown that hyperbaric oxygen mobilizes stem cells from the marrow to the wound site to direct the wound repair [46]. In addition, hyperbaric oxygen potentiates the white blood cells' ability to destroy bacteria [47]. Hyperbaric oxygen is most effective if there is adequate vascular inflow. Before undergoing hyperbaric oxygen treatment, candidates should undergo an oxygen challenge test to see whether there is a rise in the local tissue oxygen pressure after exposing the lungs to increased oxygen content. Breathing in 100% oxygen should lead to at least a 10 mm Hg rise in tissue oxygen levels around the wound site. Diving in a chamber at two atmospheres should increase the tissue oxygen level to above 300 mm Hg. Otherwise, the hyperbaric oxygen treatments unlikely to be effective.

Combining platelet-derived growth factor with hyperbaric oxygen treatments has been shown to be more effective together than either treatment alone (Fig. 21.12) [48]. Therefore, if the clinical decision is to begin hyperbaric oxygen therapy to stimulate wound healing, growth factor should probably be applied to the wound at the same time to maximize the benefits of hyperbaric oxygen. Few wound healing adjuncts have level 1 evidence of their effectiveness in healing diabetic foot wounds [36, 49]. The use of NPWT to prepare debrided diabetic foot wounds has been shown to hasten healing and decrease amputation rates [50]. The use of biologically active wound coverage has also been shown to speed up healing [44, 45, 51–54]. Although the use of hyperbaric oxygen therapy remains somewhat controversial, there is now level one evidence that it has been shown to decrease amputation rates and hasten healing [55, 56]. These should all be considered in concert with any of the above-discussed reconstructive strategies.

Closing a Wound by Delayed Primary Closure

Closing with monofilament vertical mattress sutures creates good tissue eversion along the wound's edge without requiring deeper sutures. Interrupted suture closure gives the surgeon more option when addressing seroma, hematoma, or infection. Removal of one or two of the overlying sutures rather than opening the entire closure is usually sufficient to adequately drain an underlying fluid collection. However, if deeper infection is suspected, the wound should be fully explored (Fig. 21.13). No deep sutures should be used as they potentiate infection, may trigger a foreign body response, and may give any residual biofilm a chance to reestablish itself in the wound.

Often the skin edges are too far apart to close primarily (i.e., post fasciotomy, post fracture). Gradual reapproximation of the skin edges is possible by serial operations every 2–3 days where the skin edges are approximated up to the point of blanching with horizontal mattress sutures. NPWT can be placed over the remaining soft tissue gap to help decrease the edema and make the surrounding tissue more mobile. Alternatively, skin staples can be placed at the wound edges and a vessel loop is threaded through them much like tightening shoe laces [57]. The band is tightened daily until the edges touch and then the wound can be allowed to heal by secondary intention or formally closed using vertical mattress sutures.

Skin Graft

This is the simplest of all coverage techniques with the only prerequisite being a wound with a bed of healthy granulation tissue. The superficial layer of granulation tissue is removed to ensure that there is minimal bacterial contamination/biofilm within the interstices of the granulation buds.

Preferable donor sites include the ipsilateral thigh, leg, or instep. The use of glaborous skin from a non-weight-bearing portion of the midfoot, or even the hypothenar hand, may be beneficial in preserving the specialized dermis for small plantar wounds. The size of the defect is measured to determine the amount of skin graft needed. The area needed is then drawn on the donor site. The appropriate width skin graft guide (1″, 2″, 3″, or 4″) should be used to harvest the appropriate size skin graft. The thickness of the harvest is set

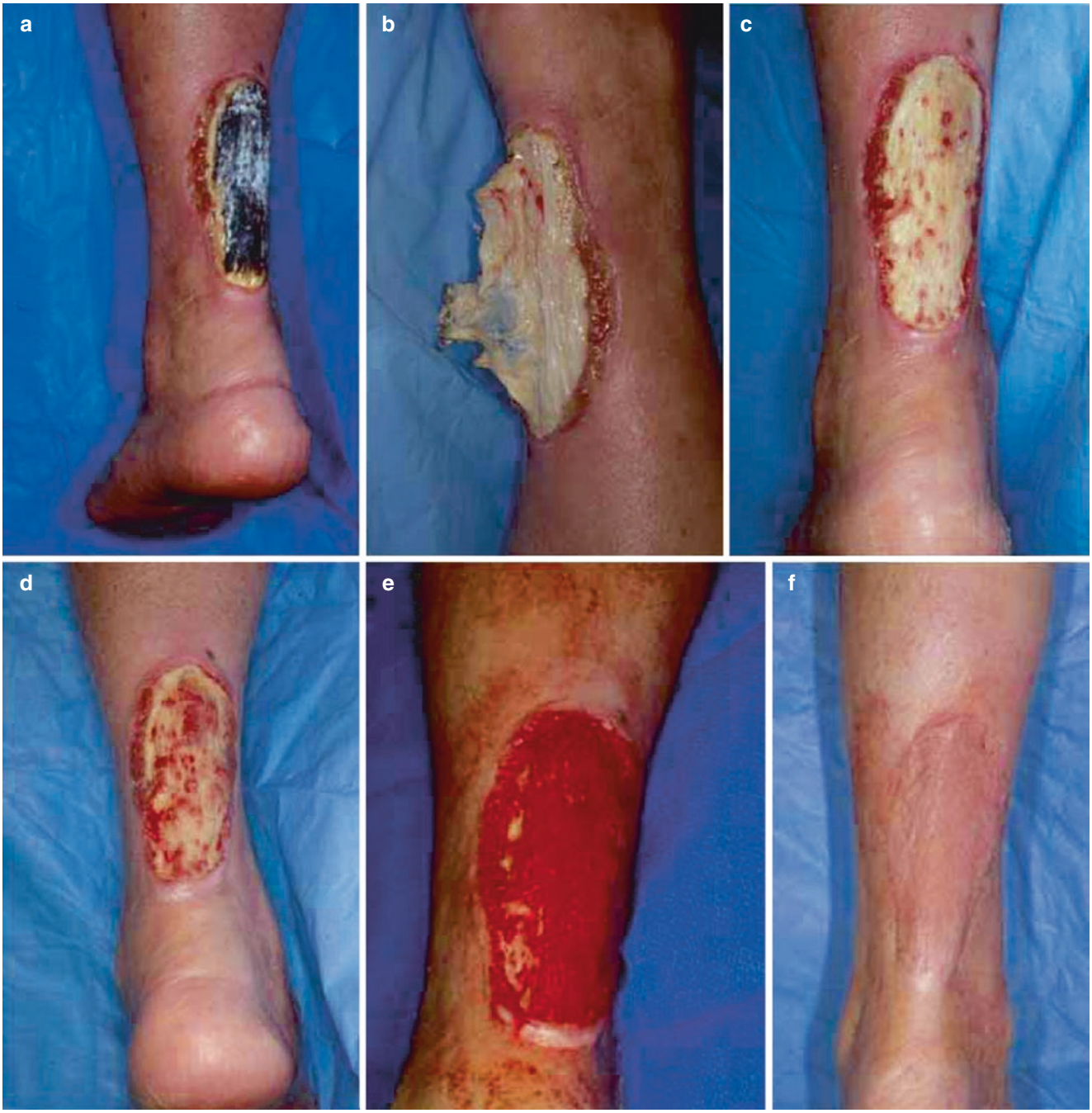

Fig. 21.12 This elderly diabetic patient presented with gangrene of the Achilles tendon (**a**). The wound was debrided by removing the loose filmy and necrotic portions of the tendon (**b**). The wound was then treated with the combination of hyperbaric oxygen and topical growth factor. Granulation appeared at week 1 (**c**), increased at week 2 (**d**), and covered the entire week by week 3 (**e**). The wound was then skin grafted for stable coverage (**f**)

at 15/1000″ which is an effective compromise between adequate take rate and skin graft contraction [58].

To prevent shearing forces from disrupting the graft, a bolster can be tied over the graft. For a bolster dressing, suture ties are placed at the edge of the wound and tied over the sponge and placed over skin graft. The bolster dressing is removed 7–10 days later (Fig. 21.14).

NPWT is an alternative method of covering fresh skin grafts and provides successful skin graft take rates of as high as 95% [17, 59]. NPWT facilitates maximal contact between the skin graft and the bed, helps stabilizes the skin graft on the bed to counteract shear forces, and removes any excess fluid that could disrupt the contact between the graft and the underlying bed (Fig. 21.15). It has been shown to be more

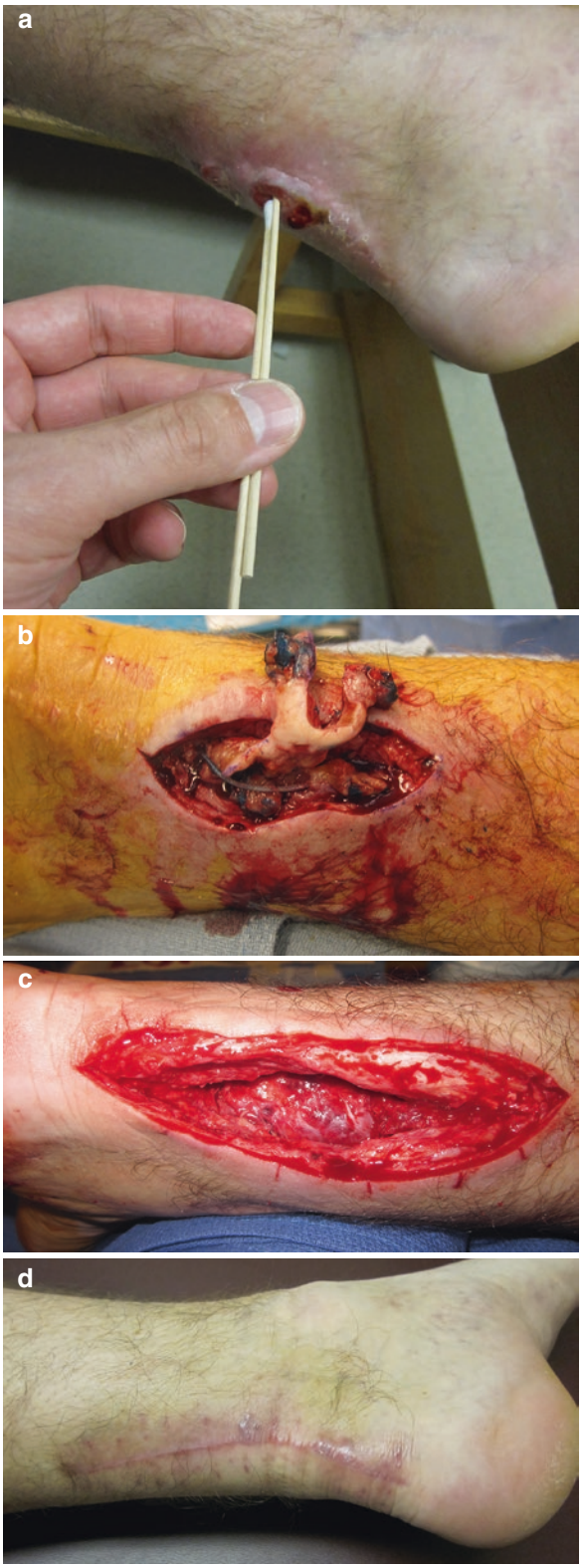

Fig. 21.13 This patient presented with a chronic draining wound for approximately 1 year following Achilles tendon repair. The cotton tip applicator is a useful tool for determining wound depth (**a**). The wound was debrided and nonabsorbable, colonized stitch material was removed (**b**). Debridement removed all indurated, nonviable, or contaminated tissue to healthy, bleeding tissue (**c**). The skin was then closed primarily with vertical mattress stitches and no deep stitches for definitive closure (**d**)

effective than bolster dressing in ensuring high initial skin graft takes [60]. The fresh skin graft is first covered with a non-adherent dressing (silicone or Vaseline mesh). The VAC sponge is then placed on top and continuous pressure is applied for 3–5 days.

When considering skin grafting over bone, tendon, or joint, creating a neodermis improves the chances of skin graft flexibility and durability. Integra® artificial dermis (Integra LifeSciences Holding Company, Plainsboro, New Jersey) is composed of an overlying removable silicone film (to prevent desiccation) with an underlying dermal matrix of cross-linked bovine collagen and chondroitin sulfate [61–63]. The dermal layer functions as a dermal template to facilitate the migration of the patient's own fibroblasts, macrophages, lymphocytes, and endothelial cells as well as new vessels. The sheet of Integra is meshed, cut to fit the wound, and affixed to the site with staples or suture. Over the ensuing week(s), a new cell populated dermis is formed. The revascularization process is accelerated two- to threefold by placing NPWT over Integra [64]. Then, the silicone layer can be removed so that a thinner skin autologous skin graft (8/1000″ to 10/1000″) can be placed on it (Fig. 21.16).

For heel wounds, an external fixator is very useful because it not only immobilizes the ankle but also suspends the foot in mid air so that the patient cannot disrupt the graft (Fig. 21.17). If the graft is on the plantar aspect of the foot, there should be no weight-bearing until the skin graft has matured (usually 6 weeks). For wounds on the weight-bearing portions of the foot (heel, lateral midfoot, under the metatarsal heads), plantar glabrous skin grafts are the ideal source of autographs because they permit the regeneration of the normal glabrous plantar surface [65].

Local Flaps

Local flaps are flaps with an unidentified blood supply adjacent to a given defect that are either rotated on a pivot point or advanced forward to cover the defect. They come in various shapes (square, rectangular, rhomboid, semicircular or bilobed) [66]. They usually consist of skin and the underlying fat or skin, fat, and the underlying fascia. They, however, can also include the muscle. It is important to carefully preplan the flap by first accurately determining the size of the defect after debridement. The flap should be designed in the area where the tissue is the most mobile. Using a template when designing the flap and holding its base at the pivot point as it is being swung into the defect is the best way of estimating the adequateness of the design. The ratio of length to width is critical for the survival of the tip of the flap [67]. Because the blood flow to the skin in the foot and ankle is not as developed as in the face, the length-to-width ratio should not exceed a 1:1 or 1:1.5 ratio. The viability of such a flap is

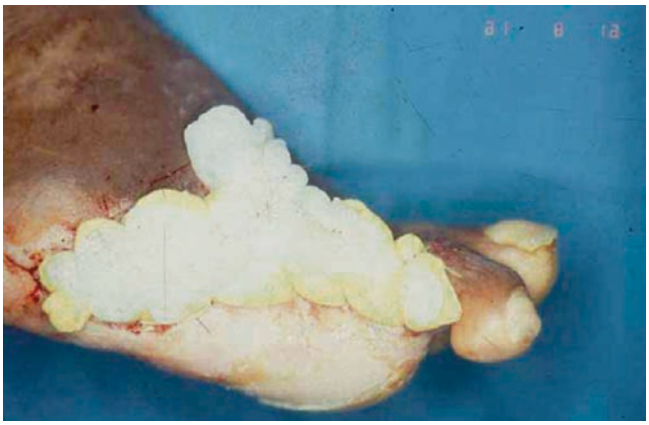

Fig. 21.14 To prevent shearing forces from disrupting the graft, a bolster can be tied over the graft. Bolster stitches are placed around the graft by going through the edge of the skin graft and wound bed, tying the suture and leaving one end long enough to then tie over the bolster. Vaseline gauze is then placed on the graft and wet un-wrung cotton balls are place on top of the gauze. The long ties are then tightly tied over the cotton balls which wrings out excess fluid as the cotton balls conform to the underlying recipient bed. The result is application of uniform pressure over the entire skin graft. The bolster dressing is removed 7–10 days later

increased when one can Doppler out a cutaneous perforator at the base of the planned flap. To ensure adequate tension free coverage, a slightly larger pattern should be used than what would anatomically be necessary. When moving the flap to cover the defect, it is important that the flap fill the defect without tension to avoid compromising the blood flow to its distal end. A force [68] of 25 mmH causes enough venous congestion for flap necrosis unless the tension is released within 4 h.

Local flaps are very useful in coverage of foot and ankle wounds because they only need to be of sufficient size to cover the exposed tendon, bone, or joint. The rest of the wound can then be covered with a simple skin graft. This combination of limited local flap and skin graft frequently obviates the need of larger pedicled or free flaps. If correctly designed, a local flap can also improve the surgical exposure of the underlying tissue if corrective surgery has to be performed [69]. The harvesting of an appropriately designed flap often improves the exposure of joints, bone, or tendons sufficiently to avoid making an extra incision. In addition, local flaps are a very useful mode of reconstruction when

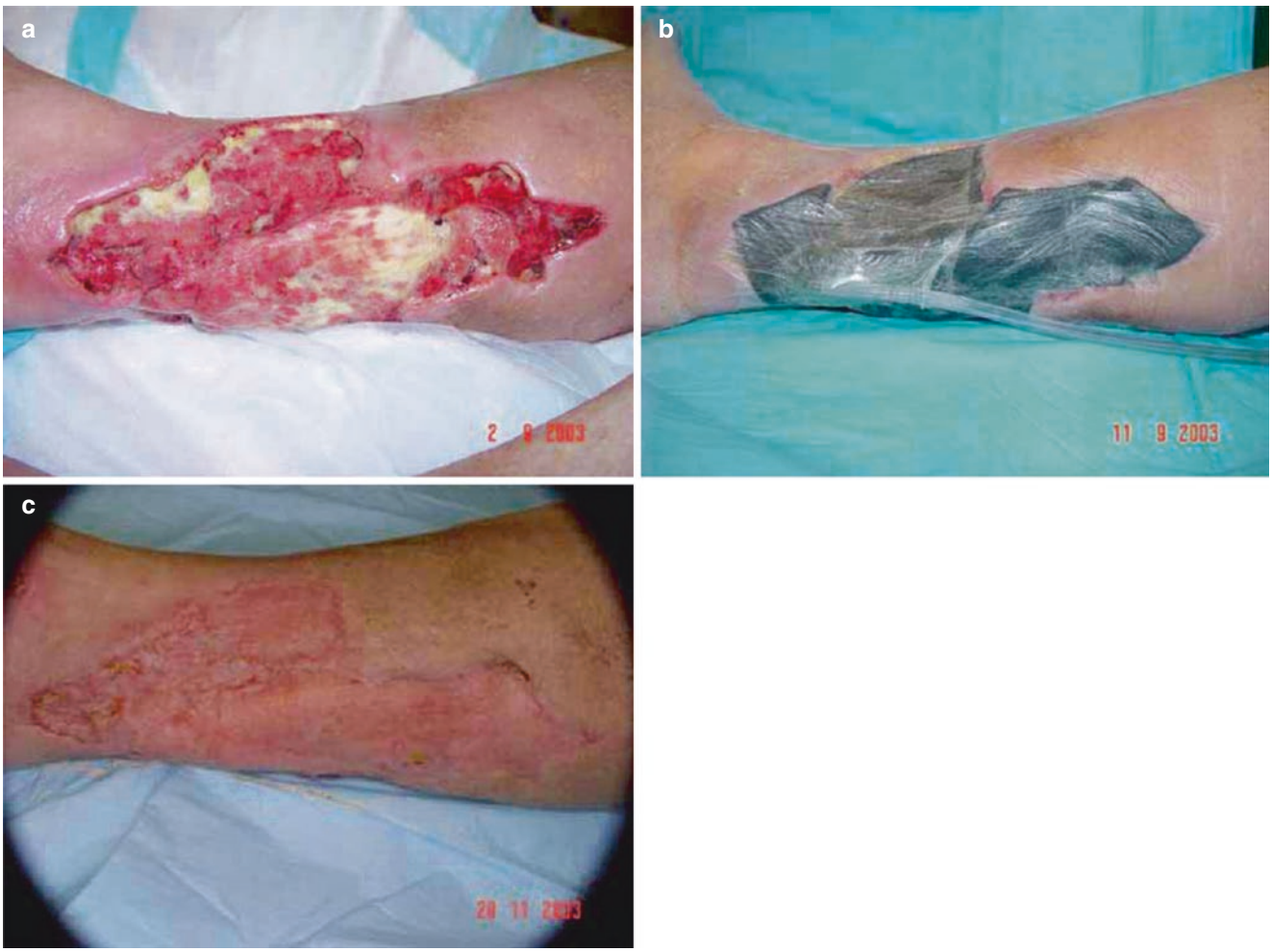

Fig. 21.15 NPWT facilitates maximal contact between the skin graft and the bed (**a**), helps stabilizes the skin graft on the bed to counteract shear forces while removing any excess fluid. The fresh skin graft is first covered with a non-adherent dressing (silicone or Vaseline mesh). The NPWT sponge is then placed on top (**b**) and continuous pressure is applied for 3–5 days postoperatively. The graft is then allowed to fully heal with a simple semiocclusive dressing such as Vaseline gauze (**c**)

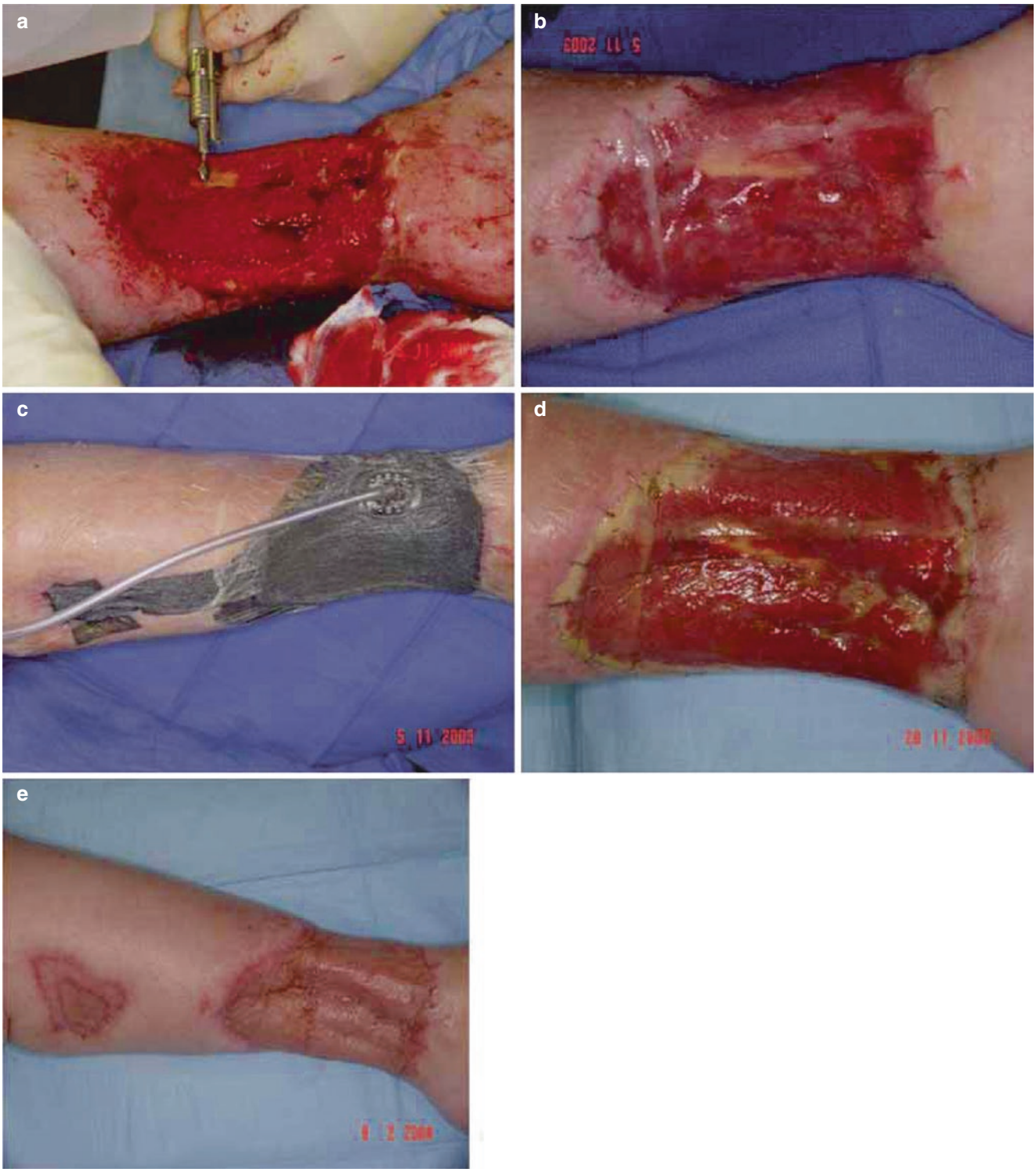

Fig. 21.16 This diabetic had exposed tibia and large almost circumferential wound just above the ankle (**a**). The sheet of Integra is meshed, cut to fit the wound, and affixed to the site with staples or suture (**b**). NPWT is placed over the Integra to speed up the vascularization of the dermal template (**c**). Once that has occurred (**d**), the silicone layer can be removed so that a thin skin autologous skin graft (8/1000″ to 10/1000″) can be placed on it. The skin graft is covered with silicone mesh and NPWT for 3–5 days and then a normal dressing is place on the graft as it continues to heal (**e**)

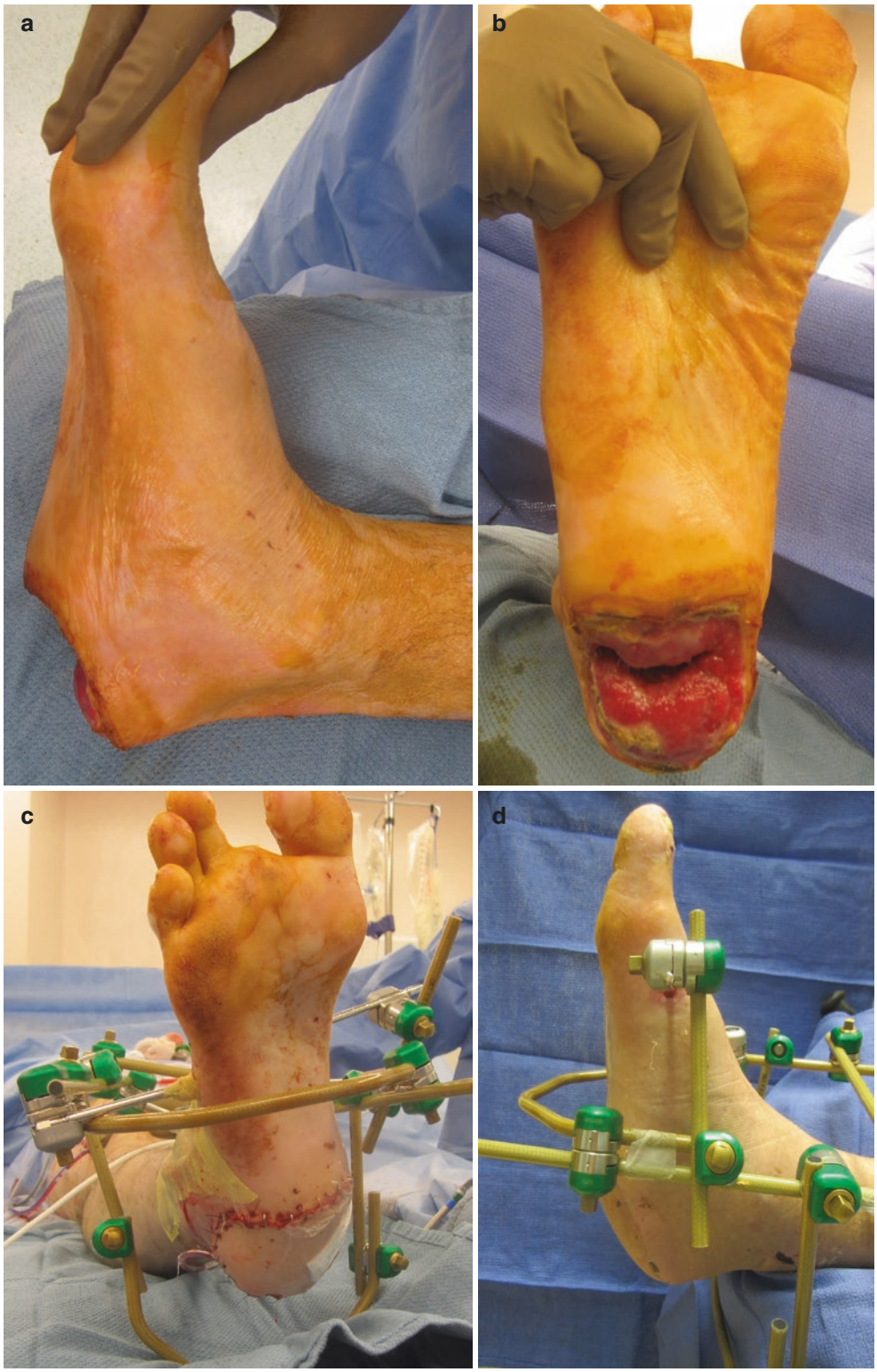

Fig. 21.17 This patient presented with a significant hindfoot wound with calcaneal osteomyelitis (**a**, **b**). Following debridement of all nonviable material and negative cultures, an anterior lateral thigh flap was utilized to obliterate dead space and resurface the hindfoot. An external fixator was placed to maintain the ankle in neutral and protect the healing reconstruction (**c**, **d**)

trying to close a wound through an Ilizarov-type fixator. This is because the frame often makes it impossible to perform the extensive dissection required for pedicled flaps or to provide the necessary space to perform the microsurgical anastomosis for free flaps.

Flaps that Rotate Around a Pivot Point

These flaps rotate around a single pivot point and therefore need to be planned carefully to avoid excessive tension along the radius of the arc of rotation. The *rotation flap* is designed when a pie-shaped triangular defect is created to remove a

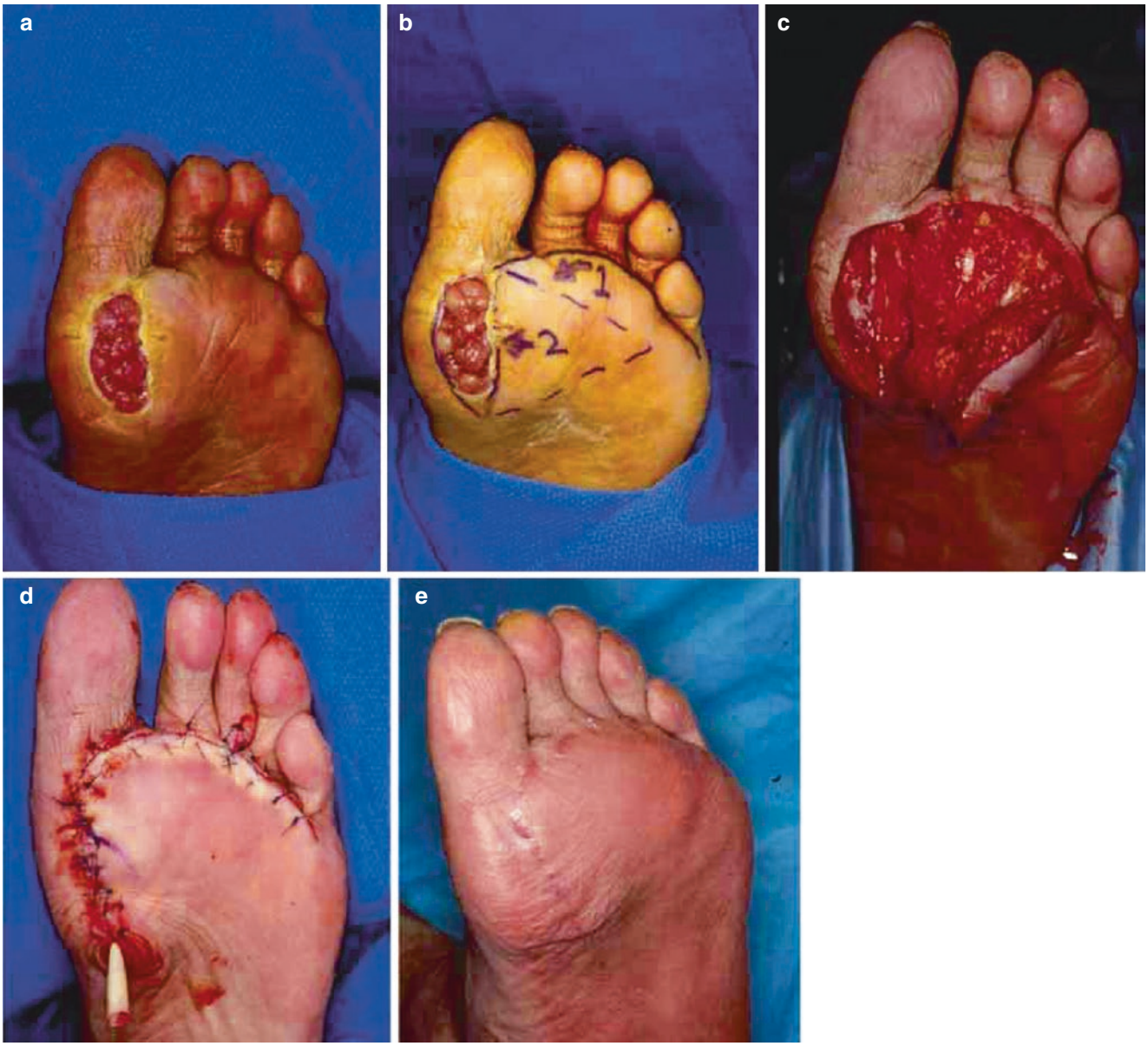

Fig. 21.18 This is a morbidly obese diabetic with an ulcer under the first MTP (**a**). Potential flaps for closure included a rotation flap and a V to Y advancement flap (**b**). The wound was debrided and a rotation flap was chosen as the mode of reconstruction. The flap was elevated with great care to preserve the neurovascular bundles to the toes (**c**). The flap is sewn into position with biased stitches to prevent tension on the distal end of the flap (**d**). The flap went on to heal despite poor compliance in keeping weight off the foot during the healing phase (**e**)

lesion or preexistent defect. The base of the triangle lies along the hypothetical circumference of a semicircular flap that can then be rotated into the defect. The most useful application of this type of flap is on the plantar aspect of the foot where the flap is elevated off the plantar fascia and rotated in position. It can also be used over the plantar forefoot (Fig. 21.18), at both malleoli and on the dorsum of the foot [70, 71]. If vascular anatomical considerations dictate, the flap can also include underlying fascia and or muscle.

Transposition flaps are flaps that can be rotated up to 90 degrees. The end of the flap has to be longer than the distance between the pivot point and the edge of the defect so that when the flap is rotated, it can fit in without tension. Preplanning the rotation with gauze or paper is the key to avoiding excessive tension on the distal end of the flap at inset. The donor site can usually be closed primarily. Otherwise, it may require skin grafting. The dog ear that results from rotating the flap should not be addressed at the initial surgery. The dog ear will usually flatten down. This is the most frequently used flap to cover the malleoli or exposed tibial-talar fusion around an Ilizarov frame (Fig. 21.19).

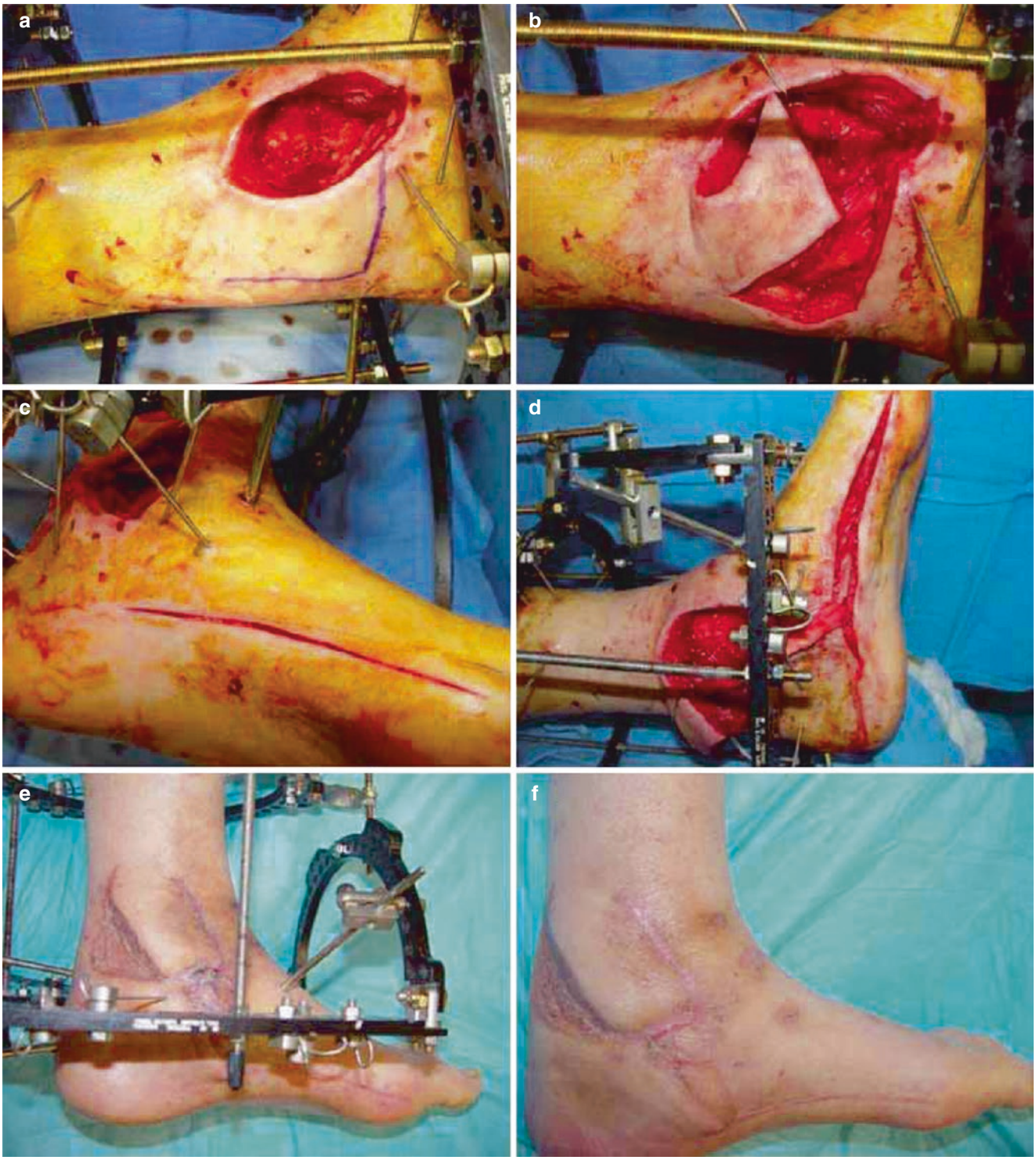

Fig. 21.19 This patient developed an infected Charcot ankle joint. The infected joint was resected and the remixing foot and ankle were stabilized using an Ilizarov frame (**a**). The defect was debrided and the upper half of the exposed joint was covered with a local flap (**b**) while the distal portion of the defect was covered with an abductor Hallucis muscle flap (**c**, **d**). The wound went on to heal without incident (**e**, **f**)

Advancement Flaps

Advancement flaps are moved directly forward to fill a defect without rotation or lateral movement. A rectangle of skin is dissected out and should include, at a minimum, skin and subcutaneous tissue. The flap is advanced into the defect. This may create a folding of the tissue at both ends of its base (burrow's triangles) which can be removed so that the skin can be sutured together without causing any irregularities in the contour. It is

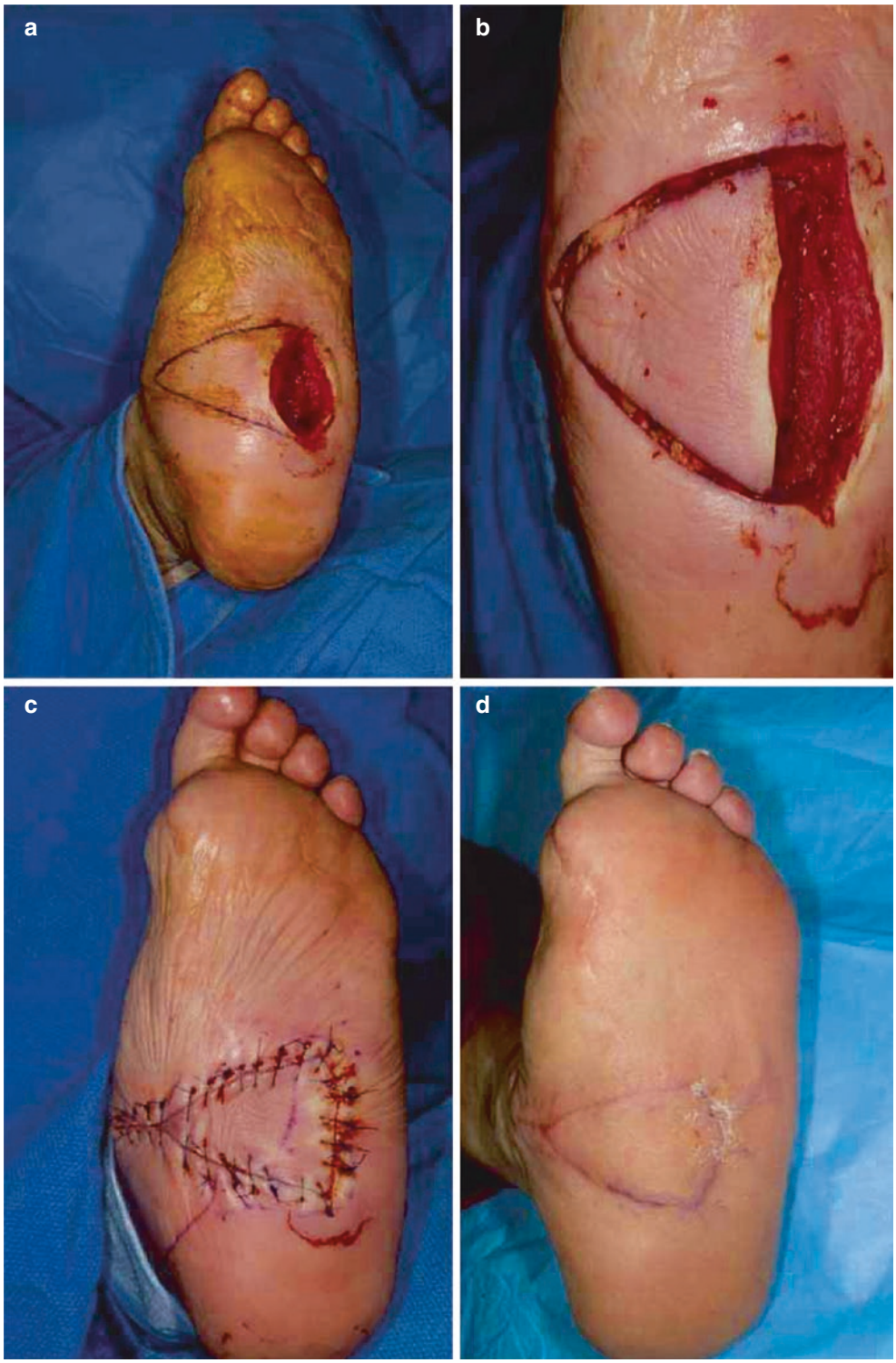

Fig. 21.20 A *V–Y flap* is a "V"-shaped flap (**a**, **b**) that, when advanced, forms a "Y" (**c**, **d**). The V–Y flap depends on direct underlying perforators to stay alive. For that reason, no undermining whatsoever can be done when dissecting out this flap. On the plantar aspect of the foot, the maximum advancement is limited to 1–2 cm

also important that the tension on the flap is adjusted so that there is no blanched area when it is in its new position.

A *V–Y flap* is a "V"-shaped flap that, when advanced, forms a "Y" (Fig. 21.20). The V–Y flap depends on direct underlying perforators to stay alive. For that reason, no undermining whatsoever can be done when dissecting out this flap. It is important to realize that the maximum advancement is limited to 1–2 cm. Therefore if the defect is larger, double opposing V–Y flaps can be used to close defects of up to 3–4 cm wide. The flap is especially useful for defects on the sole of the foot [72]. To advance the flap adequately one has to cut through the plantar fascia on both side of the triangle of tissue to be advanced. The flap should be designed as large as possible to ensure the inclusion of as many perforators as possible.

Pedicled Flaps

Pedicled flaps have identifiable blood vessels feeding the flap. They can contain various tissue combinations including cutaneous, fasciocutaneous, muscle, musculo-cutaneous, osteocutaneous, osteo-musculo-cutaneous type flaps, etc. These flaps work well if they were not involved in the initial trauma, infection, or radiation field. Otherwise, the flaps are stiff, difficult to dissect out, and difficult to transfer. In addition, the flap has to be soft and pliable because the vascular pedicle is usually intolerant of any twisting or turning that occurs when the flap is swung into its new position. Local pedicled perforator flaps, also known as propeller flaps, of the lower extremity can be useful if blood flow is present in the nearby angiosome. These flaps can be rotated up to 180° and have been studied extensively and shown to be a feasible option in many patients.

These flaps are often more difficult to dissect and may have a higher complication rate than potential free flaps [73]. Harvesting a pedicled flap often places a donor site deficit on the foot and ankle that has to be skin grafted. However, pedicled flaps allow the surgeon to perform a rapid operation with a short hospital stay that yields excellent long-lasting results. The anatomy and techniques of dissection are discussed above and in flap anatomy books [74, 75]. It is important to practice these flap on cadaver legs as the dissections are often tedious and can be difficult. The distal reach of the flap often provides insufficient tissue so that it is very important to understand the size limitations of each flap.

Lower Leg and Ankle: Muscle Flaps

The lower leg muscles are poor candidates for pedicled flaps because most are type 4 muscles with segmental minor pedicles, and therefore only a small portion of the muscle can safely be transferred. The distal portion of some of these muscles can be used to cover small defects around the ankle medially, anteriorly, and laterally [76]. For small and proximal defects, the muscle flap can usually be separated from its distal tendon to minimize the loss of function.

The Extensor Hallucis Longus m. (*anterior tibial artery*) can cover small defects that are as distal as 2 cm above the medial malleolus. The Extensor Digitorum Longus m. and Peroneus Tertius m. (*anterior tibial artery*) are used for small defects as distal as 2.1 cm above the medial malleolus. The Peroneus Brevis m. (*peroneal artery*) can be used for small defects as distal as 4 cm above the medial malleolus. The Flexor Digitorum Longus m. (*posterior tibial artery*) can be used for small defects as distal as 6 cm above the medial malleolus. The Soleus muscle (*popliteal, peroneal & posterior tibial artery*) is the only type 2 muscle in the distal lower leg where the minor distal pedicles can be safely detached and the muscle with its intact proximal major pedicles can be rotated to cover large (10 × 8 cm.) anterior lower leg defects as distal as 6.6 cm above the medial malleolus. It can be harvested as a hemi-soleus for small defects [77] and as an entire soleus for larger defects. All the just described muscles usually have to be skin grafted for complete coverage. In addition, the ankle has to be immobilized to avoid dehiscence and ensure adequate skin graft take. The use of external frames can be very useful with the former and the use of the NPWT device for the latter.

If a larger flap or wider angle of rotation is needed, one of the three major lower leg arteries with the relevant minor perforators may need be taken with the muscle flap. The sacrifice of a major artery should only be considered if all three arteries are open and there is excellent retrograde flow. These flaps are usually harvested distally and therefore the accompanying artery depends on retrograde flow. Because these flaps are larger, the tendon is also taken with the muscle. It is therefore important to tenodese the distal portion of the severed tendon to the tendon of a similar muscle so that the function is not lost. For example, if the distal Extensor Hallucis Longus (EHL) muscle is harvested, the EHL tendon distal to the harvest should be tenodesed to the Extensor Digitorum Longus (EDL) so that the hallux maintains its position during gait (Fig. 21.21). Because the loss of the anterior tibial tendon is so debilitating, the distal muscle should not be harvested unless the ankle has been or is being arthrodesed.

Lower Leg and Ankle Flaps: Fasciocutaneous Flaps

Fasciocutaneous flaps are useful for reconstruction around the foot and ankle although the donor site usually has to be skin grafted [78]. The Retrograde Peroneal flap (*retrograde peroneal artery*) [79] is useful for ankle, heel, and proximal dorsal foot defects. Its blood flow is retrograde and depends on an intact distal peroneal arterial-arterial anastomosis with either or both the anterior tibial artery and/or posterior tibial artery. The dissection is tedious and it does sacrifice one of the three major arteries of the leg. A similar retrograde anterior tibial artery flap fasciocutaneous flap (*retrograde anterior tibial artery*) has been described for coverage in young patients with traumatic wounds over the same areas. Because the anterior compartment is the only compartment of the leg whose muscle depends solely on the anterior tibial artery, only the lower half of the artery can be safely harvested as a vascular leash. The retrograde sural nerve flap [80] (*retrograde sural artery*) is a versatile neuro-fasciocutaneous flap that is useful for ankle and heel defects (Fig. 21.22). The sural artery travels with the sural nerve and receives retrograde flow from a peroneal perforator 5 cm above the lateral malleolus. The artery first courses above the fascia and then goes deep to the fascia at mid calf while the accompanying lesser saphenous vein remains above the fascia. The venous congestion often seen with this flap can be minimized if the pedicle is harvested with 3 cm of tissue on either side of the pedicle and with the overlying skin intact [81]. Problems with the venous drainage can be further helped by delaying the flap 4–10 days earlier by first tying off the proximal lesser saphenous vein and sural artery. The inset of the flap is critical to avoid kinking of the pedicle. Ingenious splinting often has to

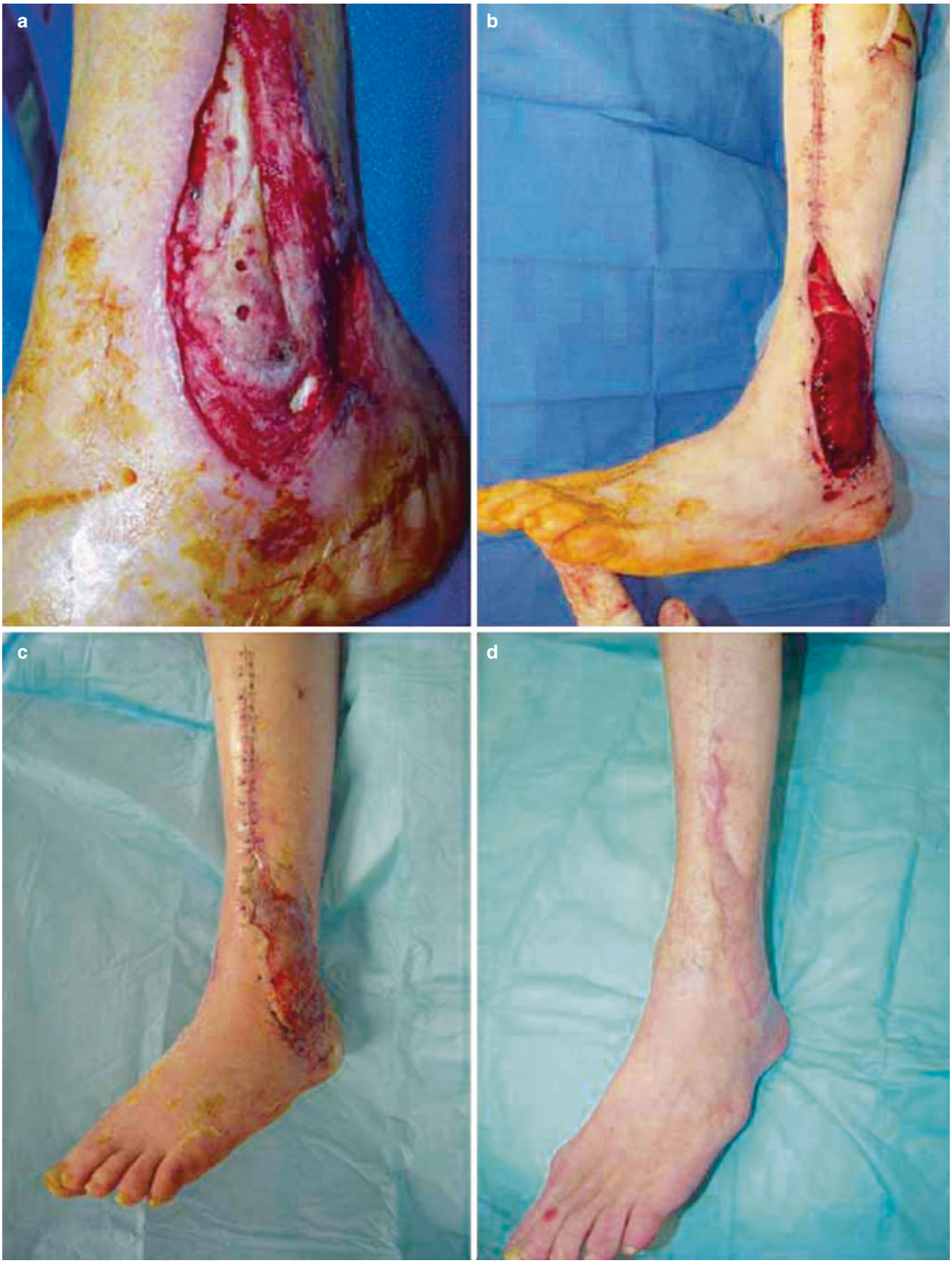

Fig. 21.21 The Extensor Hallucis Longus (EHL) muscle is harvested with the distal third of the anterior tibial artery to cover the lateral distal exposed fibula (**a**, **b**). The muscle is skin grafted (**c**). The EHL tendon distal to the harvest is tenodesed to the Extensor Digitorum Longus (EDL) so that the hallux maintains its position during gait (**d**)

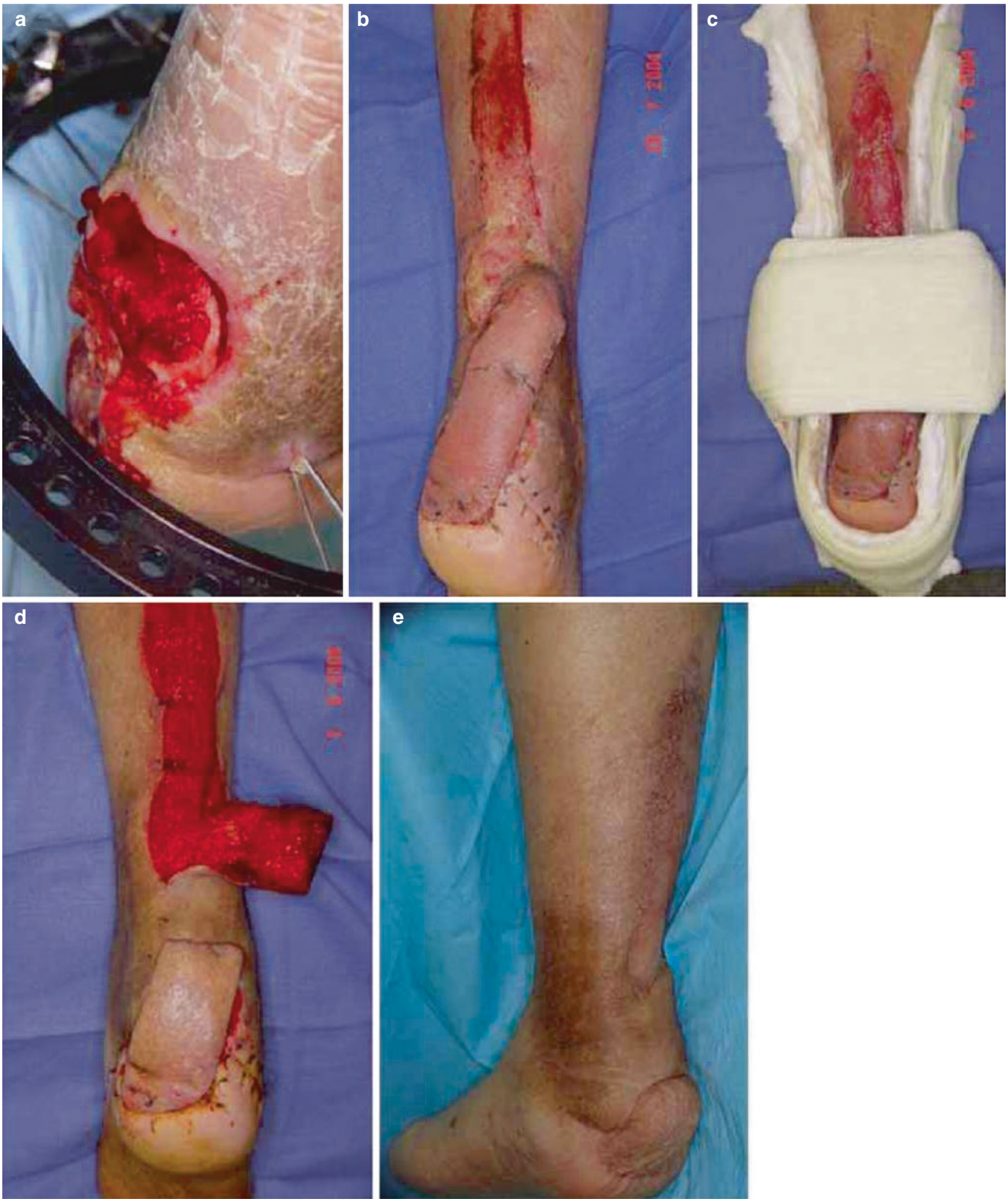

Fig. 21.22 The retrograde sural nerve flap is a neuro-fasciocutaneous flap that is useful for ankle and heel defects. The patient has a heel ulcer with osteomyelitis of the calcaneus (**a**). A sural artery flap is dissected out and inset over the defect (**b**). A cast is designed to off weight the heel (**c**). Alternatively, an Ilizarov can be applied to off weight the heel during healing. After 2 weeks, the pedicle is cut and the defect is skin grafted (**d**). The flap goes on to heal (**e**)

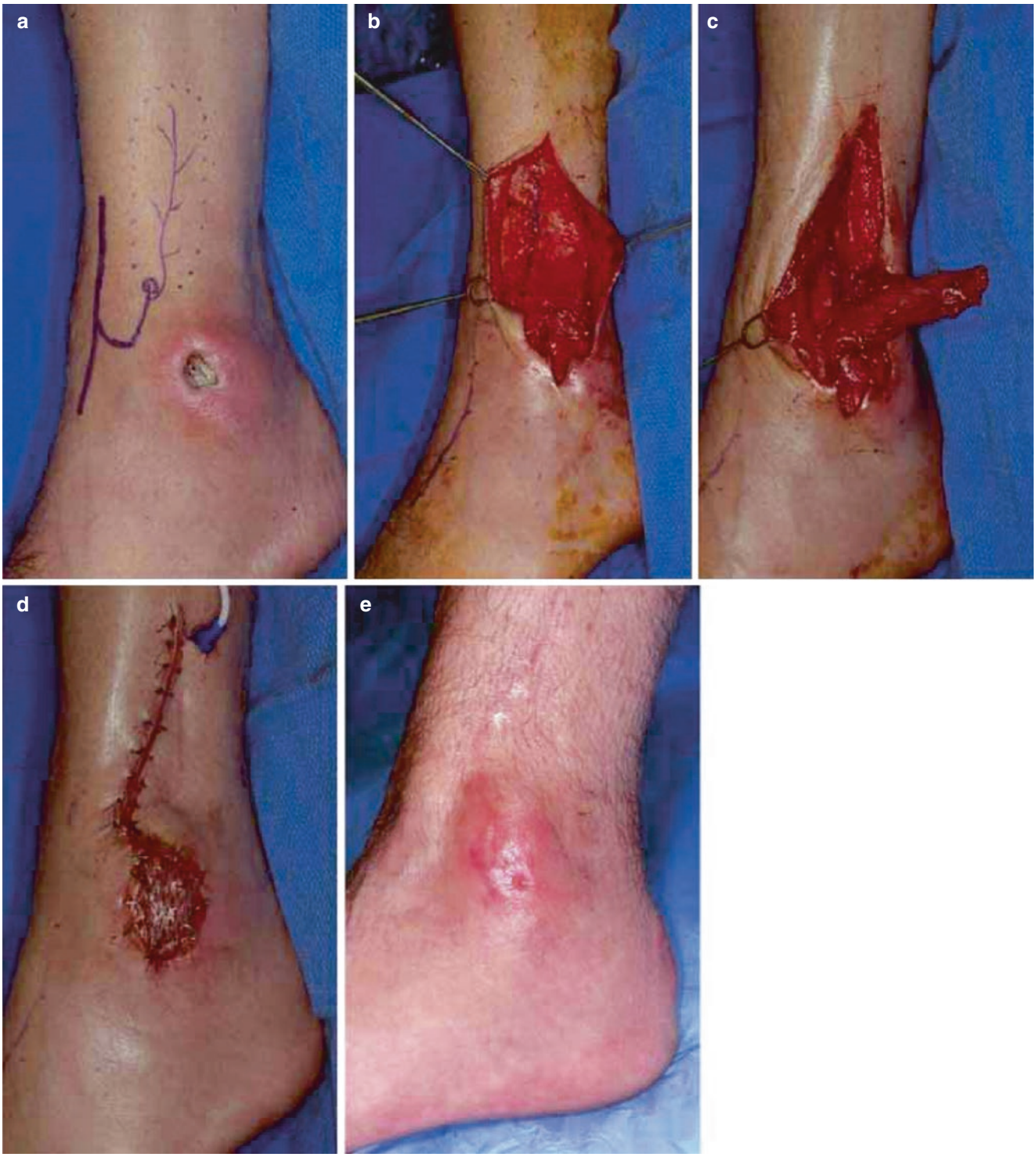

Fig. 21.23 The supra-malleolar flap based on the superior cutaneous branch of the anterior perforating branch of the peroneal artery can be used for lateral malleolar (**a**) as well as for dorsal foot defects. When harvested as fasciocutaneous flap (**b**, **c**), it is then skin grafted (**d**). Because of the new blood supply, the ulcer heals without problems (**e**)

be designed to keep pressure off of the pedicle while the flap heals (the use of the Ilizarov external frame can be very useful in this regard). The major donor deficit of the flap is the loss of sensibility along the lateral aspect of the foot and a skin grafted depression at the posterior calf donor site that may pose a problem if the patient later has to undergo a below-the-knee amputation. The supra-malleolar flap (*superior cutaneous branch of the anterior perforating branch of the peroneal artery*) can be used for lateral malleolar and heel defects as well as for dorsal foot defects (Fig. 21.23) [82]. It can be

either harvested with the overlying skin or as a fascial layer that can then be skin grafted. When harvested as a fascial layer only, the donor site can be closed primarily.

Small fasciocutaneous flaps based on individual perforators can also be designed over the row of perforators originating from the posterior tibial artery medially and the peroneal artery laterally [67]. Although the reach and size of the flap is limited, it can be expanded by applying the delay principle. These local flaps have proven to be extremely useful in the closure of soft tissue defects around the ankle in patients in an Ilizarov frame because accessibility to the normal flaps or recipient vessels may be a problem.

Foot Flaps: Muscle Flaps

The muscle flaps in the foot have a type 2 vascular pattern with a proximal dominant pedicle and several distal minor pedicles and are useful to cover relatively small local defects [73, 83]. The Abductor Digiti Minimi muscle (*lateral plantar artery*) is very useful for coverage of small mid and posterior lateral defects of the sole of the foot and lateral distal ankle (Fig. 21.24).

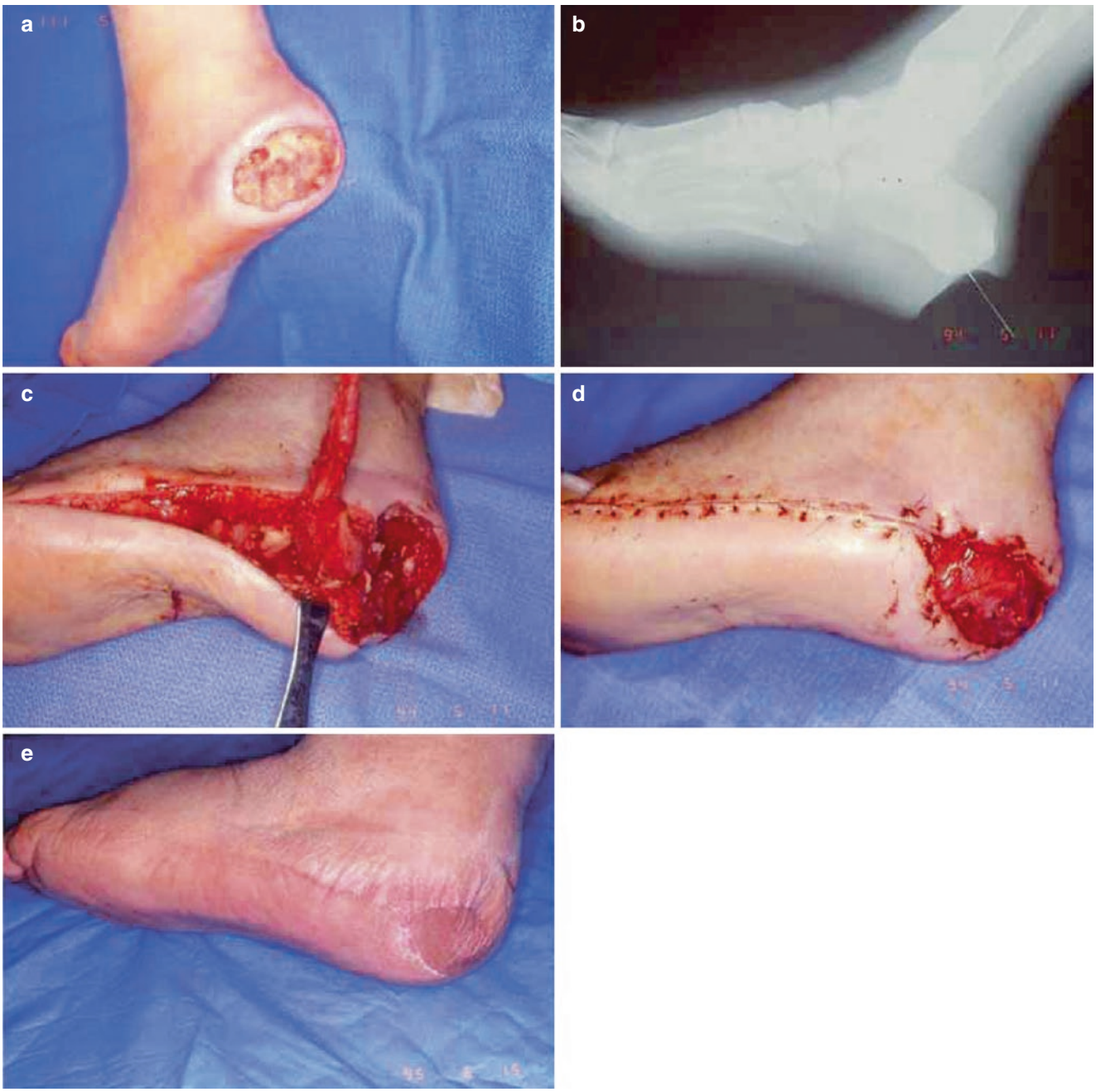

Fig. 21.24 (**a**–**e**) The Abductor Digiti Minimi muscle (*lateral plantar artery*) is very useful for coverage of small mid and posterior lateral defects of the sole of the foot and lateral calcaneal osteomyelitis. The dominant pedicle lies very close to its origin and provides sufficient blood supply so that the minor more distal pedicles can be safely ligated

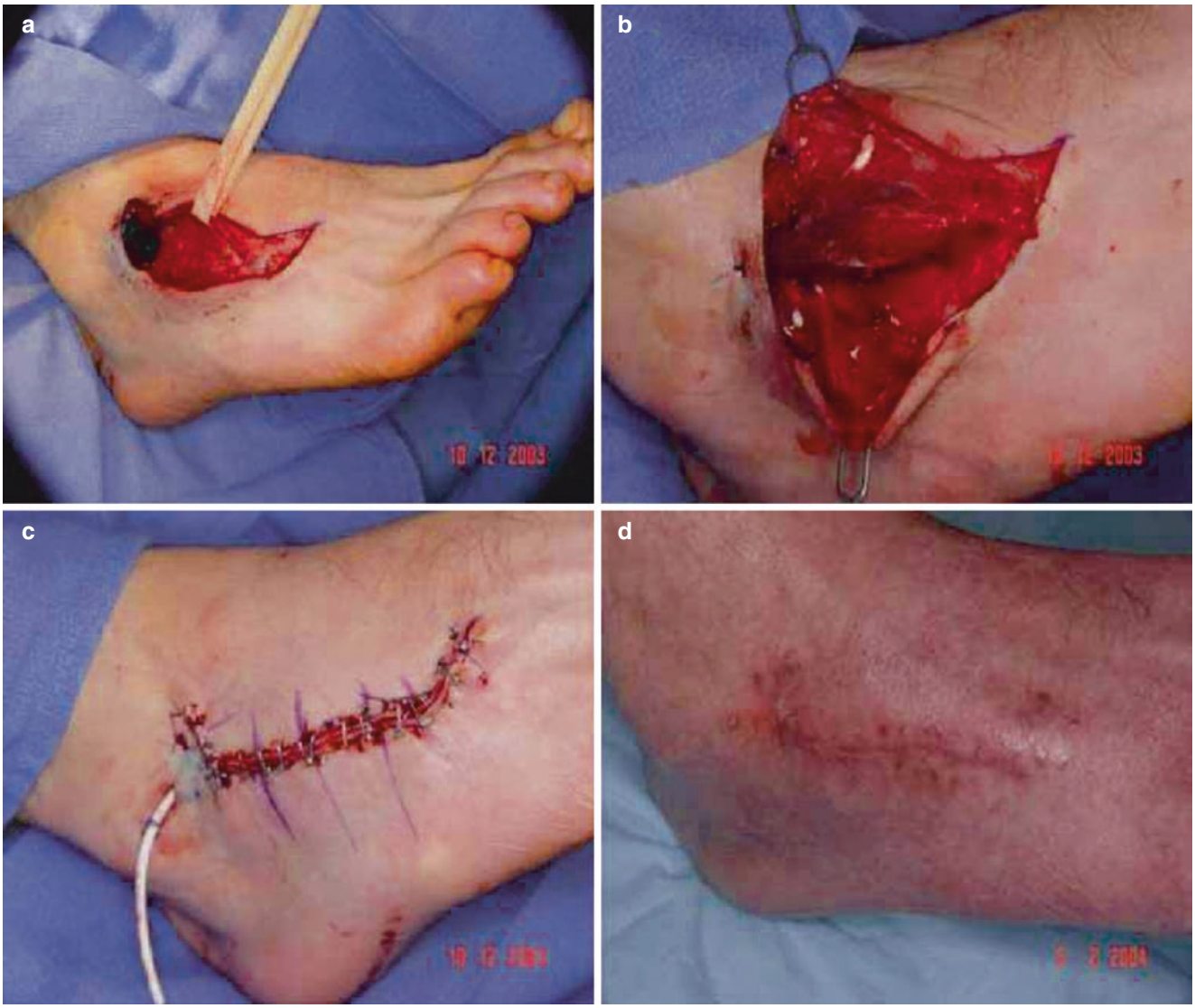

Fig. 21.25 (**a**–**d**) The Extensor Digitorum Brevis m. (*lateral tarsal artery*) has disappointingly little bulk but can be used for local defects over the sinus tarsi or lateral calcaneus. The muscle can either be rotated in a limited fashion on its dominant pedicle, the lateral tarsal artery, or in a wider arc if harvested with the distal anterior tibial artery (antegrade flow) or the proximal dorsalis pedis artery (retrograde flow)

Its dominant pedicle is just distal and medial to its origin off of the calcaneus and it has a thin distal muscular bulk [84]. The abductor hallucis brevis muscle (*medial plantar artery*) is larger and can be used to cover medial defects of the mid and hindfoot as well as the medial distal ankle (see Fig. 21.25). Its dominant pedicle is at the take-off of the medial plantar artery and its relatively thin distal muscular bulk can be difficult to dissect off the Flexor Hallucis Brevis muscle. The Extensor Digitorum Brevis m. (*lateral tarsal artery*) has disappointingly little bulk but can be used for local defects over the sinus tarsi or lateral calcaneus [85]. The muscle can either be rotated in a limited fashion (Fig. 21.26) on its dominant pedicle, the lateral tarsal artery, or in a wider arc if harvested with the entire dorsalis pedis artery. The Flexor Digitorum Brevis m. (*type 2, lateral plantar artery*) can be used to cover plantar heel defects [86]. Because the muscle bulk is small, it works best if it is used to fill a defect that can be covered with plantar tissue.

Foot Flaps: Fasciocutaneous Flaps

The most versatile fasciocutaneous flap of the foot is the Medial Plantar flap that is the ideal tissue for the coverage of plantar defects [87–89]. It can also reach medial ankle defects. It can be harvested to a size as large as 6 × 10 cm, has sensibility, and has a wide arc of rotation if it is taken with the proximal part of the medial plantar artery. It can be harvested on the superficial medial plantar artery (*cutaneous branch of the medial plantar artery*) or on the deep medial plantar artery (*deep branch of the medial plantar artery*). It is preferable to harvest the flap with the superficial branch if the artery can be dopplered because it will minimally disrupt the existing foot vascular blood supply (Fig. 21.27). However

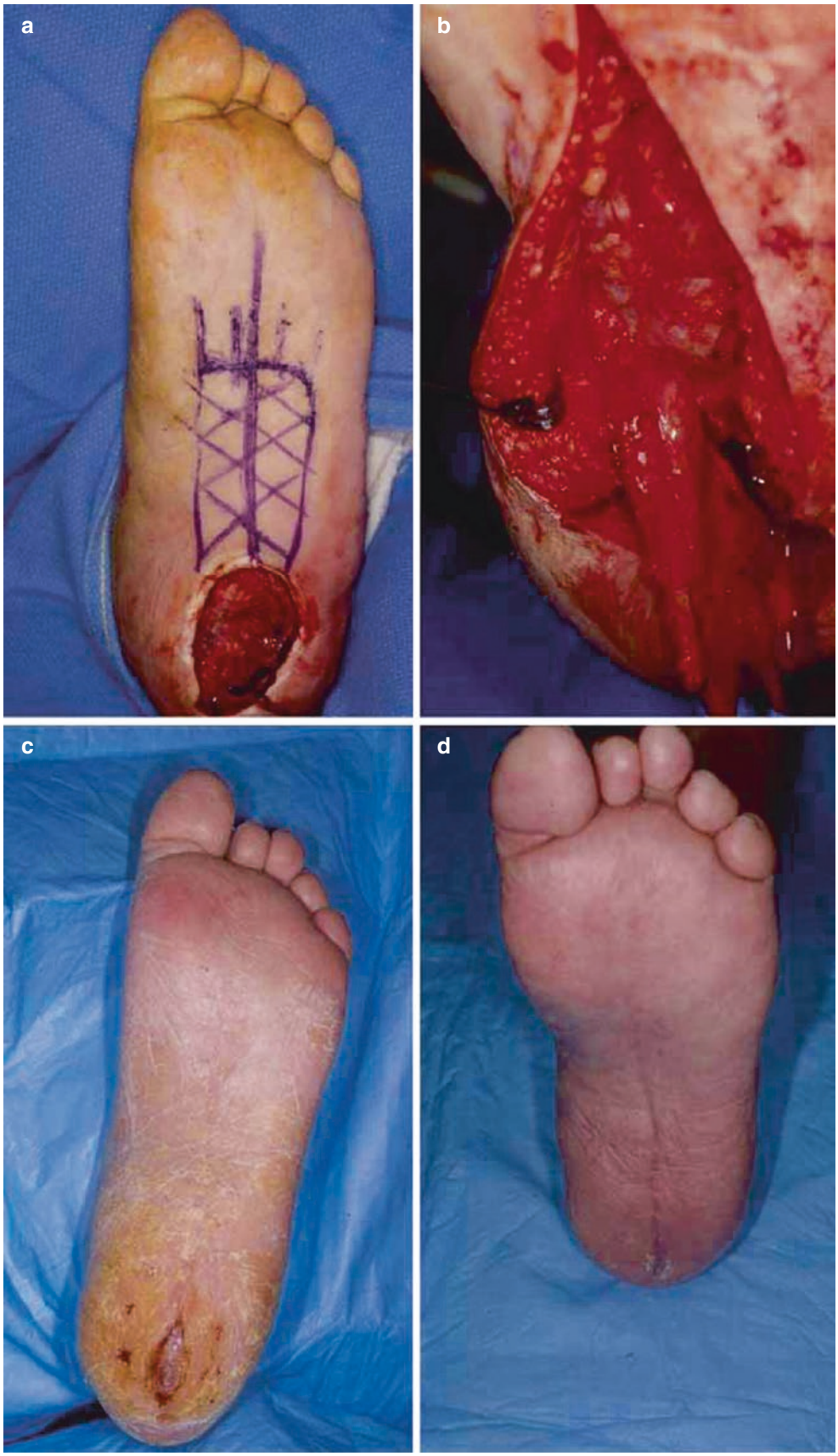

Fig. 21.26 (**a**–**d**) The Flexor Digitorum Brevis m. (*type 2, lateral plantar artery*) can be used to cover plantar heel defects. Because the muscle bulk is small, it works best if it is used to fill a defect that can be covered with plantar tissue. Skin grafting the muscle often leads to breakdown because of the lack of bulky soft tissue

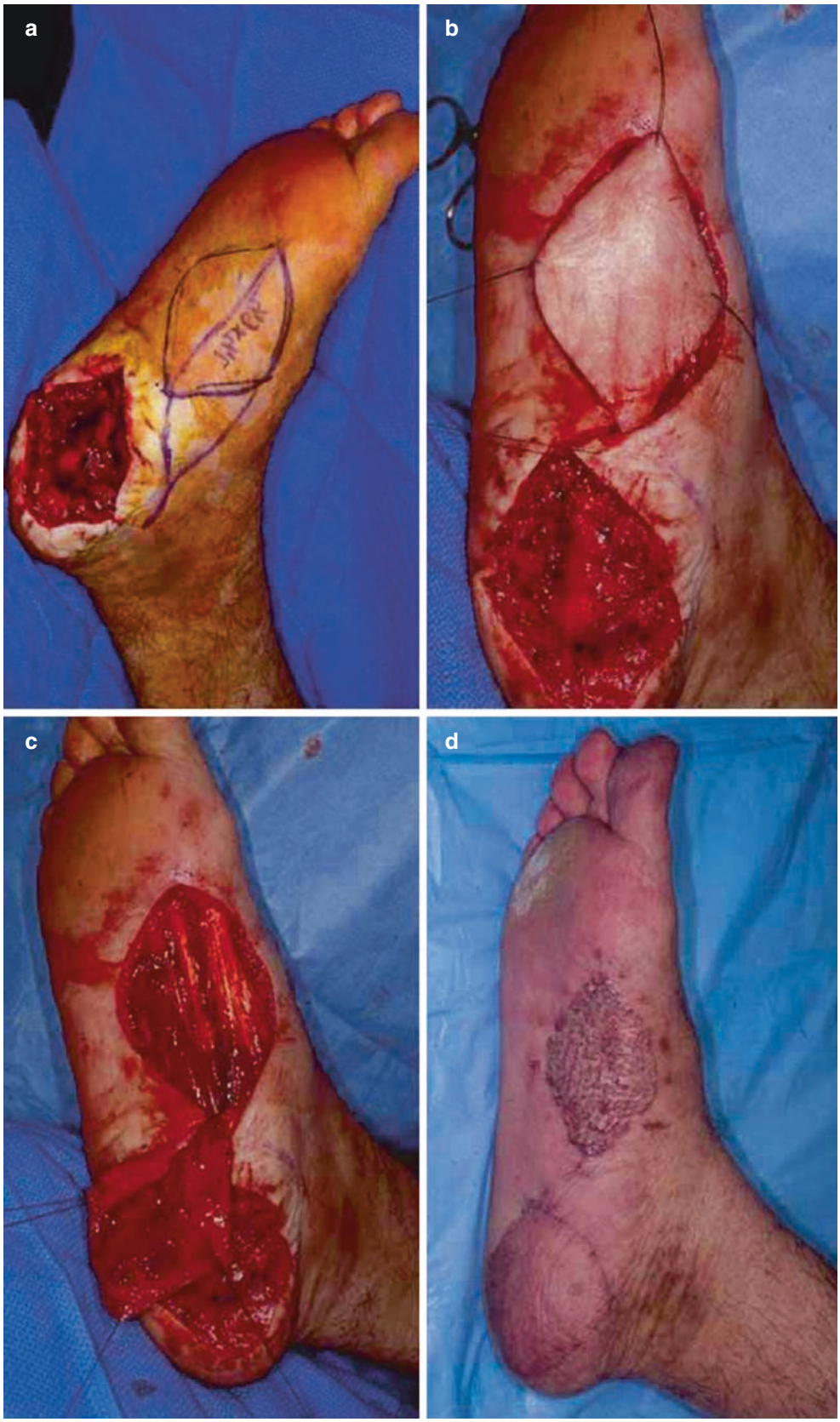

Fig. 21.27 (**a**–**d**) The most versatile fasciocutaneous flap of the foot is the Medial Plantar flap that is the ideal tissue for the coverage of plantar defects. It can be harvested on the superficial medial plantar artery (*cutaneous branch of the medial plantar artery*) or on the deep medial plantar artery (*deep branch of the medial plantar artery*). The flap below is based on the deep medial plantar artery

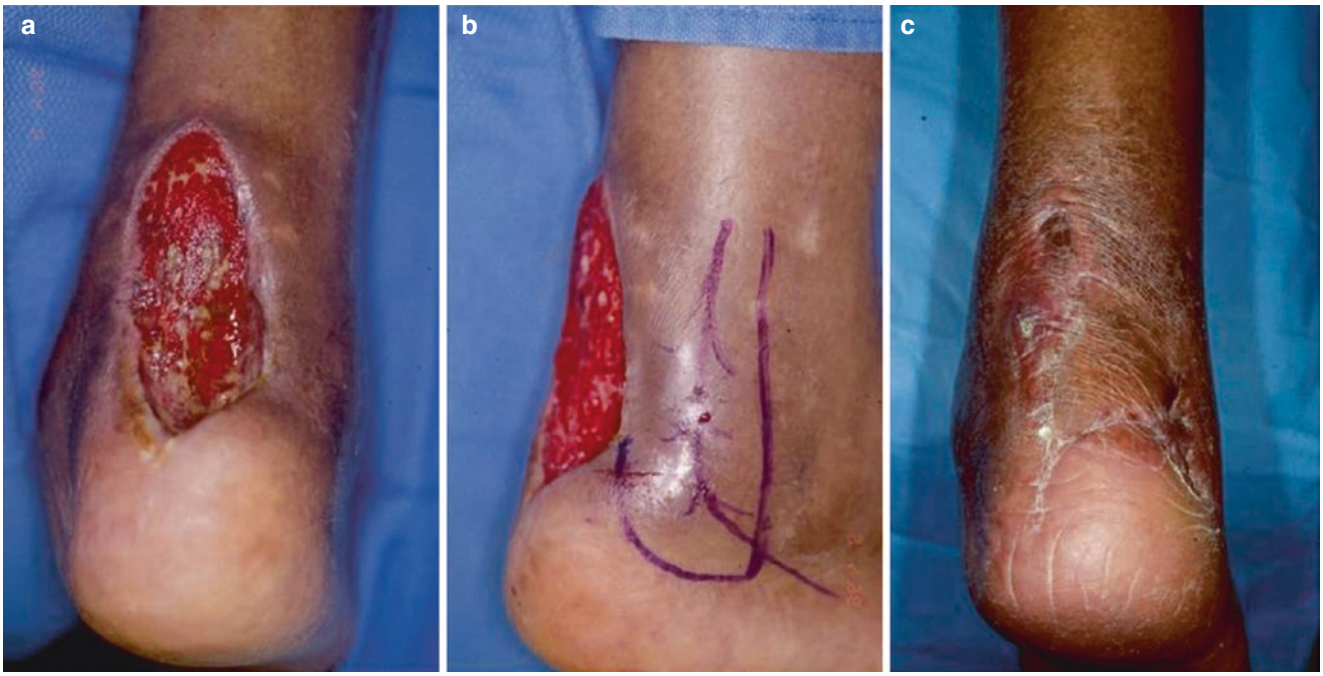

Fig. 21.28 (**a**–**c**) The Lateral Calcaneal flap (*calcaneal branch of the peroneal artery*) is useful for posterior calcaneal and distal Achilles defects. It is harvested with the lesser saphenous vein and sural nerve. Because the calcaneal branch of the peroneal artery lies directly on top of periosteum, there is a great danger of damaging or cutting it during harvest

if it is to be harvested with retrograde flow, the flap should be harvested with the deep branch of the medial plantar artery. The Lateral Calcaneal flap (*calcaneal branch of the peroneal artery*) is useful for posterior calcaneal and distal Achilles defects (Fig. 21.28) [90]. Its length can be increased by harvesting it as an "L" shape posterior to and below the lateral malleolus [91]. The Dorsalis Pedis flap (*dorsalis pedis and its continuation, the first dorsal metatarsal artery*) can be either proximally or distally based for coverage of ankle and dorsal foot defects [92]. A flap wider than 4 cm usually requires skin grafting on top of extensor tendon paratenon which leaves the dorsum of the foot with less than ideal coverage that may frequently prove to be unstable resulting in exposed tendon or even bone. The loss of the dorsalis pedis can pose problems unless the collateral circulation is intact. Because the donor site is vulnerable both from a vascular and tissue breakdown perspective, this flap is now rarely used. The filet of toe flap (*digital artery*) is useful for small forefoot web space ulcers and distal forefoot problems although the reach of the flap is always less than expected [93]. The technique involves removal of the nail bed, phalangeal bones, extensor tendons, flexor tendons, and volar plates while leaving the two digital arteries intact (Fig. 21.29). A variation of this is the very elegant Toe Island flap where a part of the toe pulp is raised directly over the ipsilateral digital neurovascular bundle [94, 95]. The flap is then elevated with its long vascular leash to cover a distal defect. The vascular leash is buried under the intervening tissue.

Complications Associated with Pedicled Flaps

A limitation to consider when utilizing local tissue is that in the event of a failed reconstruction, either biomechanical, infectious or other, additional coverage or even amputation may be necessary. In the latter circumstance, then it may be prudent to avoid flaps that may compromise the slavage operation, or the patient's total functional ability following such an operation.

Hematoma creates pressure on the flap which can limit venous return and eventually can lead to flap necrosis. The presence of free blood in the deep space is also cause for concern because the red blood cells themselves release superoxide radicals [96] that can contribute to flap necrosis. Hematoma can be prevented by meticulous hemostasis, topical agents, and closed suction drainage. Postoperatively, it is important to visualize the flap and if there is any suspicion of existing hematoma, then the wound should be explored and the hematoma evacuated. If the flap was closed with interrupted sutures, removal of one or two stitches allows for evacuation of the hematoma without risking the disruption of the whole repair. It is important to flush the space with normal saline to remove any remaining hemolyzed blood. If the hematoma cannot be removed in this way, the patient should be returned to the O.R. for formal evacuation. External pressure by applying a bandage so that it does not allow for normal postoperative soft tissue swelling or too tightly can also impede blood flow.

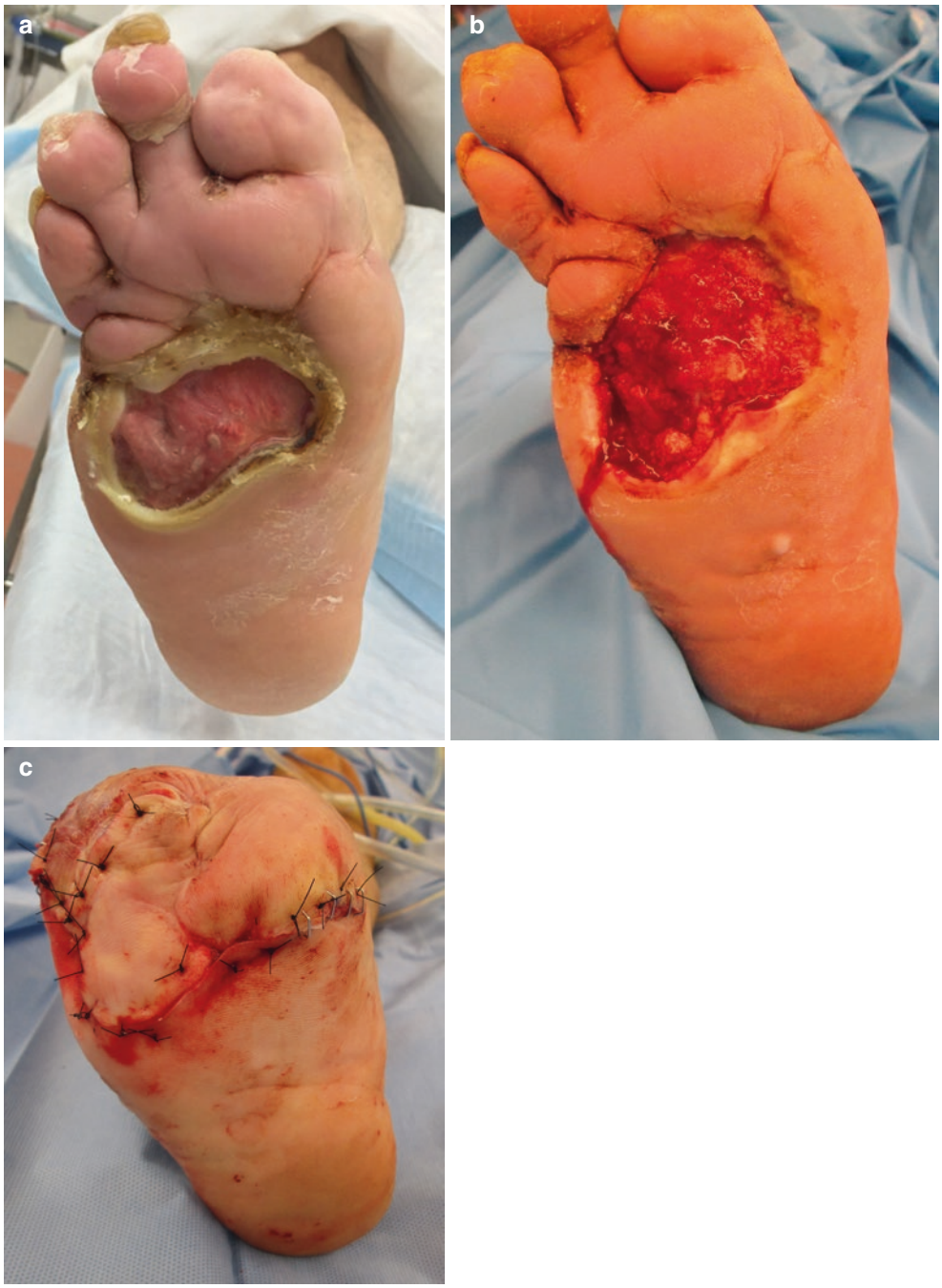

Fig. 21.29 Patient with a chronic midfoot wound and pan-metacarpal head resections (**a**). Following debridement of all nonviable, chronic tissue a large plantar wound remained, and was covered with fillet flaps from the first and second toes (**b**, **c**)

Infection can damage or destroy a flap by increasing the metabolic demand of the flap so that it outstrips existing blood supply and occluding the capillary bed which short-circuits vascular flow and leads to arterial occlusion. It is therefore important not to plan a reconstruction before all signs of infection are gone. This means that the skin edges are soft with no surrounding induration or erythema, that the pain has diminished, that there is minimal drainage, and that there are signs of healing (granulation and neo-epithelialization). This may require serial debridements that may take up to 1 month before the wound is ready.

Microsurgical Free Flap

Free tissue transfer should be considered for diabetic foot defects in patients with (1) well-controlled blood glucose levels; (2) compliance with planned postoperative restrictions; (3) minimal or well-controlled cardio-pulmonary comorbidities; (4) an extremity that is deemed better than amputation; and (5) the potential to return to an ambulatory or improved functional status following flap closure. This includes large hindfoot wounds (>6 cm), defects in patients devoid of the posterior tibial vessels (from either trauma or disease), or patients who have been revascularized to the dis-

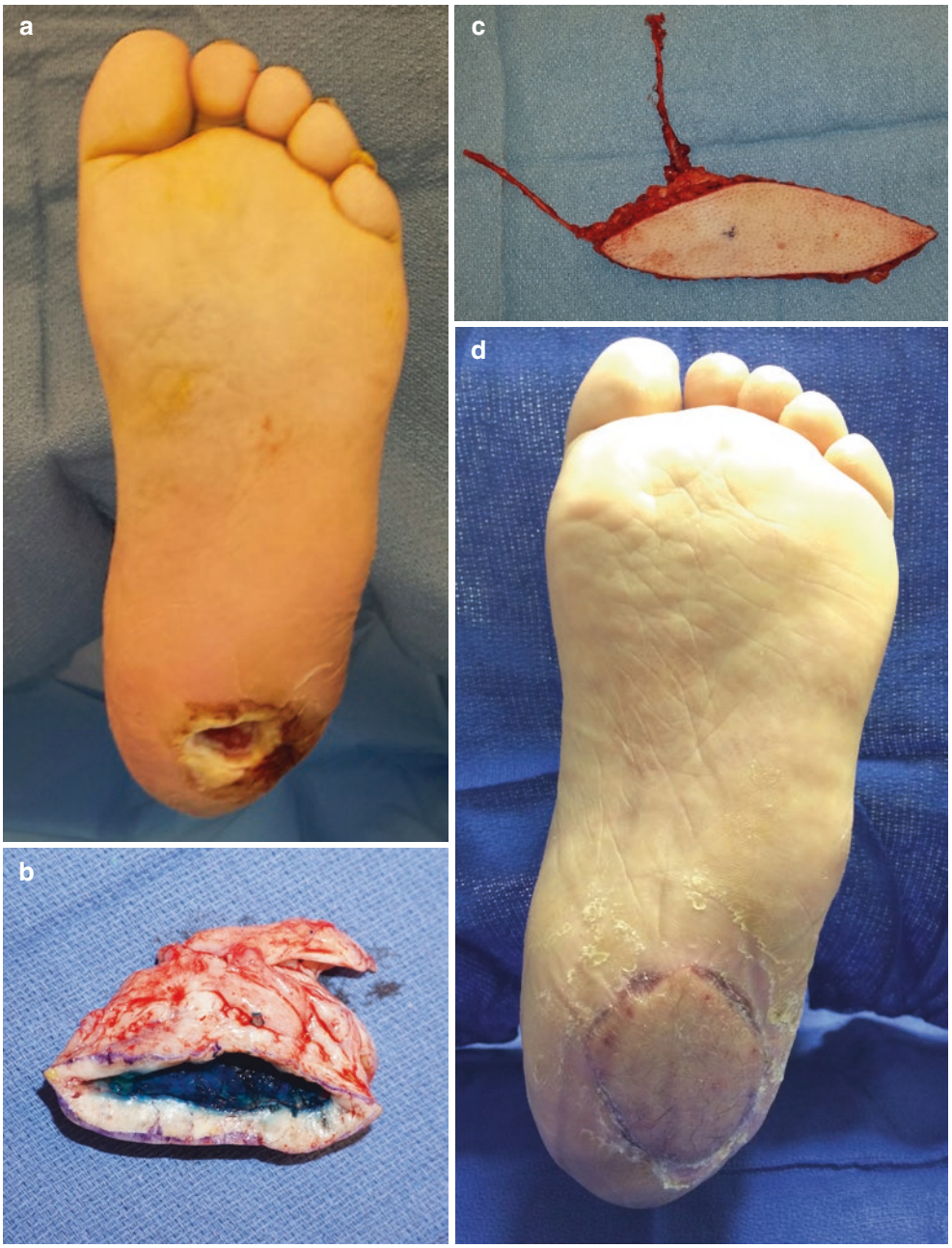

Fig. 21.30 This 32-year-old type 1 diabetic had a chronic calcaneal wound despite offloading and debridement (**a**). Methylene blue was utilized to paint the wound and ensure complete debridement of chronically exposed tissue (**b**). An anterior lateral thigh (ALT) flap was harvested with a branch of the lateral femoral cutaneous nerve (LFCN) for a sensate reconstruction (**c**). The ALT provided stable coverage with adequate soft tissue, following an end-to-side anastomosis with the posterior tibial artery and coaptation with the medial calcaneal nerve (**d**, **e**)

tal anterior tibial/dorsalis pedis artery via bypass grafts. With the exception of the sural artery flap, all of the regional flaps described for hindfoot repair require antegrade blood flow in the posterior tibial artery and its branches (medial and lateral plantar arteries). Patients with wounds on an ischemic angiosome without targeted direct revascularization are candidates for free flap coverage.

Most foot wounds with bone and/or tendon exposure are best reconstructed with "thin" free flaps. Thin skin flaps or fascial flaps surfaced with a skin graft will provide durable, thin cover that is aesthetically pleasing and permits normal shoe wear. Some skin flaps may also be sensate if harvested with the sensory nerve that supports the flap (Fig. 21.30). They can include vascularized tendon or bone for specific reconstructive tasks. Flaps that have proven to be very successful include the radial forearm flap, the lateral arm flap, the parascapular or dorsal thoracic fascia flap, and the anterolateral thigh flap.

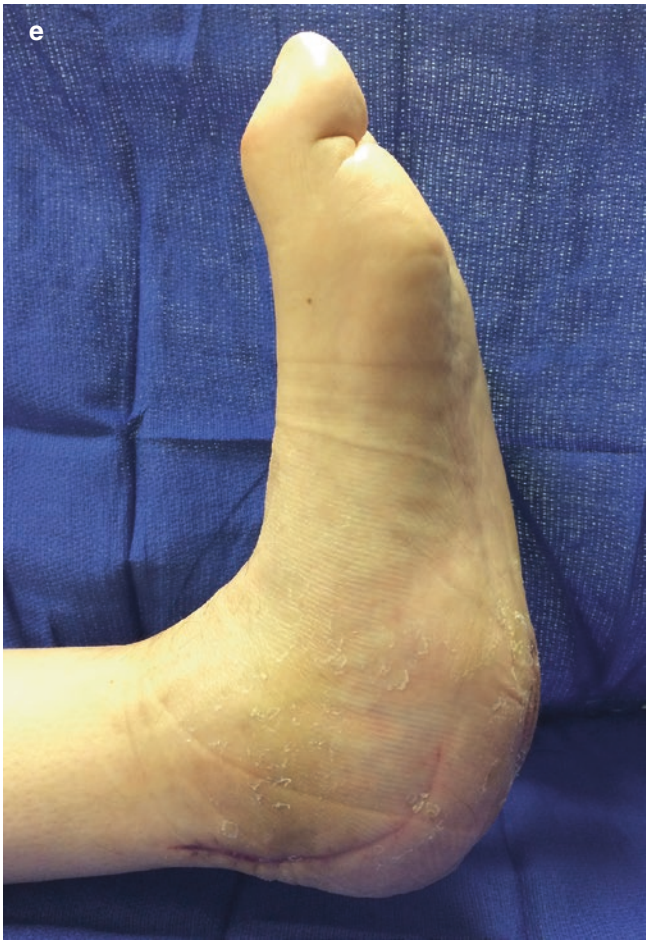

Fig. 30 (continued)

The parascapular flap based on the circumflex scapular artery is a good choice for large defects [97, 98]. The increased dermal thickness of the back skin may be more robust or resilient to repetitive trauma when compared to a thinner dermis such as the radial forearm flap. Alternatively, because the flap is supplied by an axial vessel that courses along the length of the flap, thinning is typically performed at the superficial level as opposed to deep, to avoid injury to the pedicle. Colen et al. have described an adaptation of the flap where only fascia with a thin layer of fat is harvested [99], that is then skin grafted to yield a thin flap.

The lateral arm flap based on the posterior radial recurrent vascular pedicle was first described by Katseros et al. [100]. It is a sensory flap (lower lateral cutaneous nerve of the arm) with a relatively long vascular pedicle (up to 14 cm). Including the skin overlying the elbow can extend the flap size.

The radial forearm flap is an excellent option for dorsal foot wounds [101, 102]. The advantage of the radial forearm flap is that it is thin, pliable, and can be harvested with a sensory nerve (the lateral antebrachial cutaneous nerve). The palmaris longus tendon can also be used to reconstruct missing extensor tendons on the dorsum of the foot if necessary. Prior to harvesting the flap, adequate inflow to the hand must be verified with an intact vascular arch, as removal of the radial artery creates a dependency upon the ulnar and interosseous arteries. As such, in patients with active fistulas, subclavian stenosis, or ischemic wounds on the fingers or hand, this flap should be approached cautiously. The radial forearm flap is also very useful around the malleoli. The radial artery with the venae comitantes provides an excellent vascular pedicle up to 14 cm in length. The flap, if inset properly at the time of flap transfer, rarely needs tailoring. The donor site is skin grafted, and apart from the obvious resulting color disparity, is very manageable.

The anterolateral thigh (ALT) flap is based upon perforators originating from the descending branch of the lateral circumflex femoral system [101]. Originally described by Song [102] and popularized by Koshima [103, 104], the anterior lateral thigh flap is well accepted and can supply a large amount of subcutaneous fat and skin on a safe and reliable pedicle with minimal functional donor site morbidity. The flap may be raised both sensate with the lateral femoral cutaneous nerve, and as a flow through flap [105]. Thinning

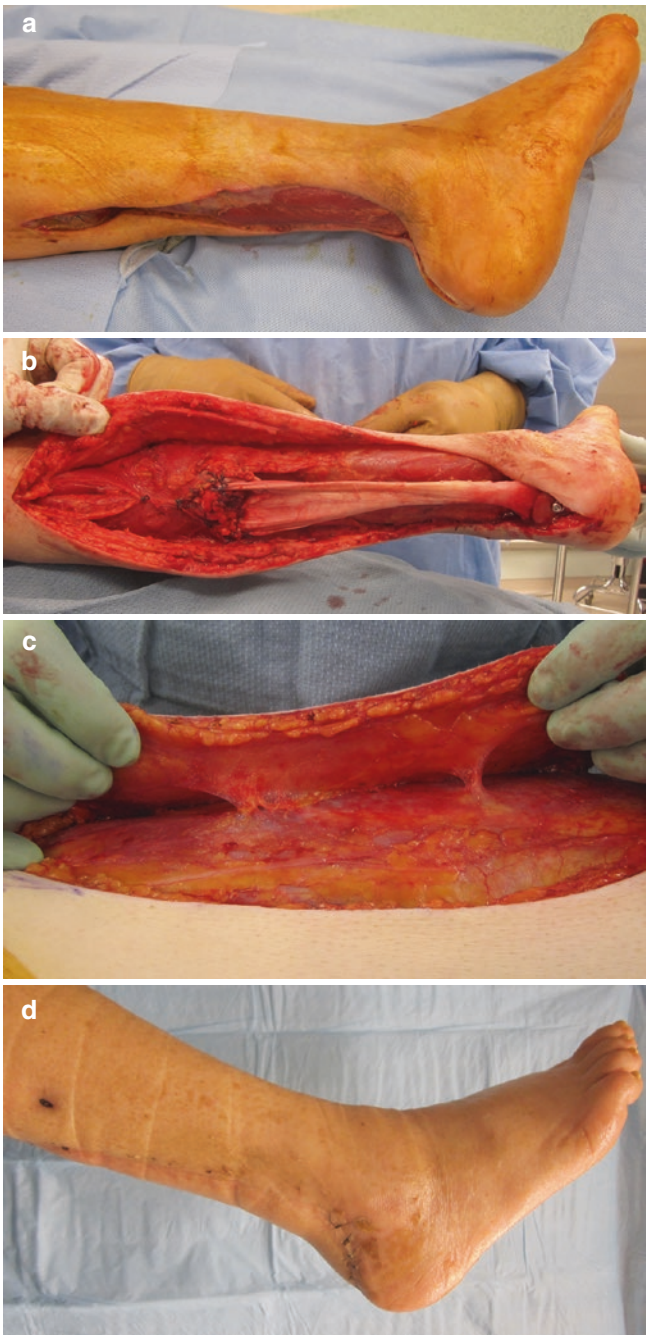

Fig. 21.31 This posterior leg wound resulted in complete loss of the superficial posterior compartment and Achilles tendon (**a**). An Achilles tendon allograft and calcaneal bone block was utilized for reconstruction, with a superthin ALT flap. The ALT flap was harvested at the level of scarpa's fascia, and two perforators are noted exiting the flap before penetrating the crural fascia (**b**, **c**). The thin flap and Achilles allograft allowed for independent ambulation with normal shoe gear (**d**)

of the flap is well tolerated even to the level of the subdermal plexus for tailoring to a particular defect [106] (Fig. 21.31). The anatomy and dissection of ALT flaps has been well established for both head and neck and lower extremity defects [107]. Additionally, in the setting of an ascending

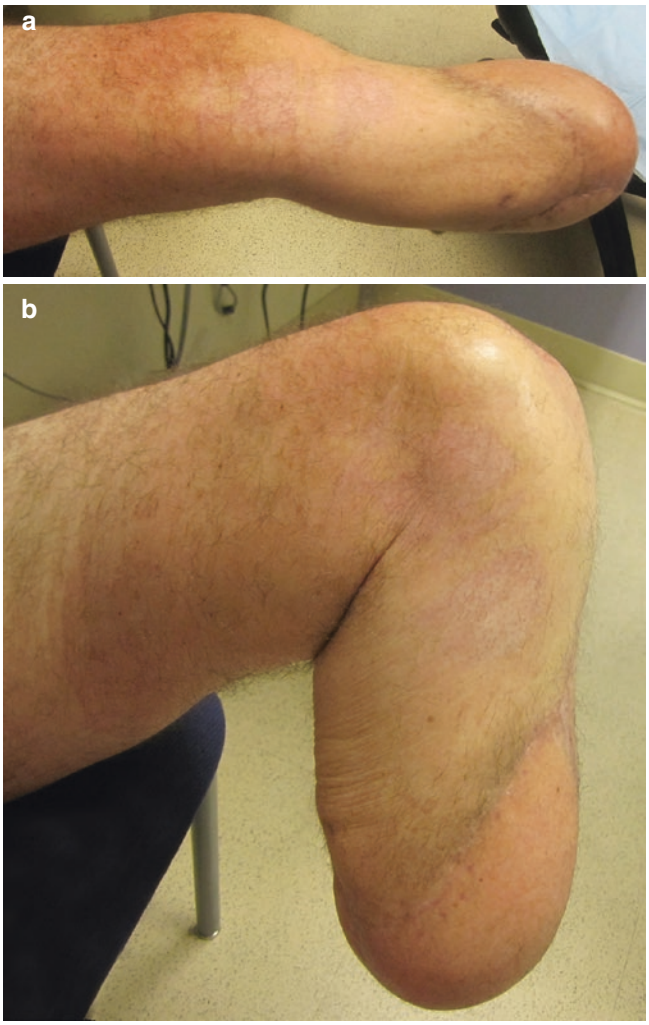

Fig. 21.32 Below knee amputation stump distally covered with an anterior lateral thigh flap to preserve length (**a**). The stump maintains excellent motion and contour to allow the patient to ambulate with a prosthesis (**b**)

infection or massive tissue loss following necrotizing fasciitis, microsurgical transfers can be performed to maintain limb length for a functional prosthesis. If proximal soft tissue is limited but healthy bone remains, an improved functional outcome may be facilitated by coverage with a thin, pliable flap such as the ALT (Fig. 21.32).

Pedicled instep flaps are frequently used in weight-bearing plantar reconstruction, but may not be available after severe foot injuries. Gaining popularity is the contralateral sensate free instep flap for reconstruction of a defect with "like tissue" [108]. This flap yields both excellent functional and aesthetic long-term results.

As in other locations, the use of muscle to obliterate dead space and aid in delivering both neutrophils and parenteral antibiotics to regions of chronic osteomyelitis is particularly important [109, 110]. The most frequently harvested muscles include the gracilis and serratus anterior muscles (Fig. 21.33).

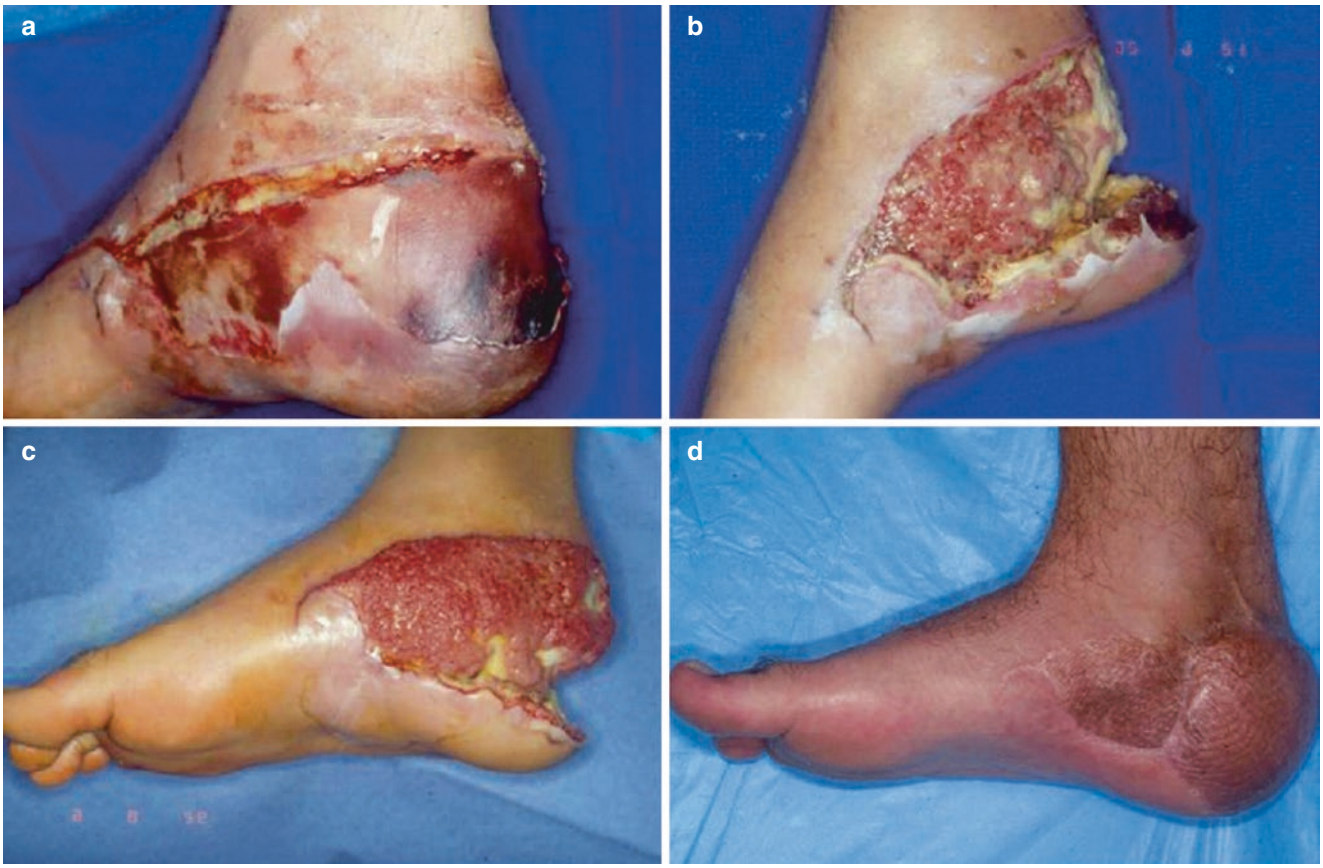

Fig. 21.33 This diabetic patient had an avulsion injury over the right heel (**a**). The wound was serially debrided (**b**) until a healthy base wound healing base was achieved (**c**). A serratus muscle flap with skin graft was used to over the heel. This is the appearance of the foot 5 years later (**d**)

A word of caution with respect to the use of the latissimus dorsi flap: while this flap is an attractive option in many other parts of the body, the functional loss of the latissimus dorsi must be taken into account in lower extremity reconstructions where many of these patients will be crutch or wheelchair dependent for a prolonged amount of time. There is still some debate whether muscle plus skin graft versus fasciocutaneous flaps on the plantar surface of the foot holds up better under the stress of ambulation. As the denervated muscle flap will atrophy, it can create an excellent and robust flap that needs minimally secondary contouring. This may also help the muscle flap obliterate dead space or tunneling easier [118, 119]. The rectus abdominus muscle flap is very useful because it is easy to harvest, has an excellent pedicle, and is a thin broad muscle. If the muscle is stretched over the recipient site, it may be made even thinner. Caution should be utilized, however, in those patients that may ultimately require higher level amputations, especially bilateral, as loss of abdominal strength may markedly decrease the patient's ability to sit up or perform transfers as the negative donor site morbidity of harvesting the rectus muscle on overall core strength, hernia and bulge has been studied extensively. The gracilis muscle is also an excellent choice for foot and ankle reconstruction. It should be harvested from the ipsilateral leg. This limits all incisions to the same extremity. The pedicle is somewhat smaller and shorter than that of the rectus abdominus muscle. Free muscle transfers to the foot tend to swell, which makes it more difficult to fit the foot into a shoe. To minimize this swelling, several technical maneuvers may be helpful. First, the outflow should be optimized by performing two vein anastomoses. To minimize the profile of the flap, it should be inset under tension so that it lies flat and at the same height as that of the surrounding tissue. After the flap has survived and the skin graft has healed, compression therapy helps in improving the overall contour. Stockings with at least 30 mm Hg should be worn by the patient. If that is insufficient, the muscle may need debulking.

Alternatively, as some fasciocutaneous flaps are innervated they could potentially be more effective on the sole of the foot in non-neuropathic patients. Fasciocutaneous flaps are ideal for providing skin coverage while preserving underlying tendon motion. With many of the described flaps, a neurorrhaphy may be performed to improve reinnervation. Outcomes and overall benefit of this added procedure remain controversial. Some studies report no difference in flap survival or ulceration rates between innervated and non-

innervated flaps. Regardless of age or attempted nerve coaptation, patients with neuropathies or heavy scarring due to chronic wounds may not show improvements in flap reinnervation when compared to surrounding tissue [111].

May and others reviewed their experience with patients who underwent free muscle transplantation and split-thickness skin grafting to the weight-bearing portions of the foot and concluded that cutaneous sensibility did not appear to be necessary to maintain a functional and well-healed extremity [112]. In a similar report, Stevenson and Mathes [113] also noted successful coverage of a weight-bearing plantar defect after use of microvascular transplantation of muscle with skin graft coverage. Levin and colleagues reviewed the Duke experience with free tissue transfer to the lower extremity and presented a subunit principle to foot and ankle reconstruction [114]. The authors noted that bulky or ill-conceived flap designs may interfere with proper shoe fitting and prevent efficient ambulation. For plantar reconstructions, flaps that developed late ulceration were more likely to include a cutaneous paddle, with the breakdown usually occurring at the flap/glaborous skin junction. Levin advocates cutting the edge of the flap and the glaborous skin obliquely to maximize the interface surface area as this may decrease the effect of shearing forces. True to all free flap reconstructions, importance should be placed on meticulous flap inset, removal of underlying bony prominences, patient education, and frequent follow-up.

Reconstructive Options by Location of Defect

Forefoot Coverage

Toe ulcer or gangrene is best treated with a limited amputation that uses all remaining viable tissue so that the amputated toe is as long as possible when closed. The surgeon should attempt to at least preserve a sufficient portion of the proximal phalanx to act as a spacer preventing the adjacent toes from drifting into the empty space. If the hallux is involved, attempts should be made to preserve as much length as possible because of its critical role in ambulation [115]. A toe island flap from the second toe is an excellent way to fill a defect on the hallux without having to resort to shortening it.

Ulcers under the metatarsal head(s) occur because biomechanical abnormalities place excessive or extended pressure on the plantar forefoot during the gait cycle. Although hammer toes are contributing factors and should be corrected, the principle cause of the abnormal biomechanical forces is usually a tight Achilles tendon that prevents ankle dorsiflexion beyond the neutral position. A percutaneous release of the Achilles tendon is performed if both portions of the Achilles tendon are tight while a Gastrocnemius recession is performed if only the Gastrocnemius portion of the Achilles tendon is tight. With the release of the Achilles tendon, forefoot pressures drops dramatically and the ulcer(s), if it does not involve bone, should heal by secondary intention in less than 6 weeks [116, 117]. This decrease in push-off strength persists and prevents recurrent ulceration by over 50% over the next 25 months [9, 10].

The complications associated with the gastrocnemius recession are far less than those associated with the percutaneous Achilles tendon release. The primary complication of a gastrocnemius recession is a hematoma from tears in the underlying soleus muscle. On the other hand, an overaggressive percutaneous release of the Achilles tendon leads to over lengthening and subsequent calcaneal gait and eventual plantar heel ulcers (13–14%) that are extremely difficult to heal. Healing may require retightening the Achilles tendon or ankle fusion in addition to treating the ulcer.

For patients with normal ankle dorsiflexion who have a stage 1–3 plantar ulcer under the metatarsal head due to a plantarly prominent metatarsal head, the affected metatarsal head can be elevated with preplanned osteotomies and internal fixation. The metatarsal head is thus shifted 2–3 mm superiorly. Upward movement with its attendant pressure relief is usually sufficient for the underlying ulcer to heal by secondary intention. There should not be any transfer lesions to the other metatarsal heads because the anatomic metatarsal head parabola will be preserved. However, if the metatarsal head has osteomyelitis, it should be shaved or resected. The ulcer should heal by secondary intention if all weight is kept off the forefoot while it heals. The small deep forefoot ulcers without an obvious bony prominence can also be closed with a local flap: a filleted toe flap, a toe island flap, a bilobed flap, a rotation flap, a Limberg flap, or a V–Y flap. For larger ulcers where the metatarsal head has been resected, consideration should be given to ray amputation. Resecting the more independent first or fifth metatarsal causes less biomechanical disruption than the second, third, or fourth metatarsal because the middle metatarsals operate as a cohesive central unit.

All efforts should be made to preserve as much of the metatarsals as possible if more than one is exposed because they are so important to normal ambulation. Local tissue is often insufficient to do this in the forefoot and therefore a microsurgical free flap should be considered. If ulcers are present under several metatarsal heads or if a transfer lesion from one of the resected metatarsal head to a neighboring metatarsal has occurred, consideration should be given to doing a pan-metatarsal head resection. This is performed with two or three dorsal incisions and great care is taken to preserve the proportional lengths of each metatarsal so that the normal distal metatarsal parabola is preserved. Removing the metatarsal heads while leaving the flexors and extensors

to the toes intact helps prevent the inevitable equino-varus deformity that accompanies loss of the distal extensors.

If more than two toes and the accompanying metatarsals heads have to be resected, then a transmetatarsal amputation [118] should be performed. The normal parabola with the second metatarsal being the longest is preserved. All bone cuts should be made so that the plantar aspect of the cut is shorter than the dorsal one. If the extensor and flexor tendons of the fourth and fifth toe are intact, they should be tenodesed with the ankle in the neutral position. This helps prevent the subsequent equino-varus deformity from the loss of extensor forces that usually leads to breakdown under the distal fifth metatarsal head. If the Achilles tendon is tight, it should be lengthened [119]. As much plantar tissue as possible should be preserved so that the anterior portion of the amputation consists of healthy plantar tissue. When there are existing medial or lateral defects, the plantar flap should be appropriately rotated to cover the entire plantar forefoot. Dog ears should be resected so that the distal end is as normally tapered as possible and easy to fit into a shoe with a simple orthotic and filler.

A proximal forefoot amputation is the Lisfranc amputation where all the metatarsals are removed [120]. The direction of the blood flow along the dorsalis pedis and lateral plantar arteries should be evaluated. If both have antegrade flow, then the connection between the two can be sacrificed. However if only one of the two vessels is providing blood flow to the entire foot, the connection has to be preserved. To prevent an equino-varus deformity, one can either address the anterior tibialis tendon or the Achilles tendon. The anterior tibial tendon can be split so that the lateral half is inserted into the cuboid bone. Alternatively, the Achilles tendon has to be lengthened. The Lisfranc amputation can be closed with volar or dorsal flaps if there is sufficient tissue. If there is not adequate tissue for coverage, a free muscle flap with skin graft should be used. Postoperatively, the patient's foot should be placed in slight dorsiflexion until the wound has healed.

Midfoot Coverage

Defects on the medial aspect of the sole are non-weight-bearing and are best treated with a skin graft. Ulcers on the medial and lateral plantar midfoot are usually due to Charcot collapse of the midfoot plantar arch. If the underlying shattered bone has healed and is stable (Eichenholtz stage 3), then the excess bone can be shaved via a medial or lateral approach while the ulcer can either be allowed to heal by secondary intention or can be covered with a glabrous skin graft or a local flap. For small defects, useful local flap include the V to Y flap, the rotation flap, the bilobed flap, the rhomboid flap, or the transposition flap. If a muscle flap is needed, a pedicled Abductor Hallucis flap medially or an Abductor Digiti Minimi flap laterally works well. For slightly larger defects, large V–Y flaps, random large medially based rotation flaps or pedicled medial plantar fasciocutaneous flap can be successful. Larger defects should be filled with free muscle flaps covered by skin grafts. Great care should be taken to inset the flap at the same height as the surrounding tissue. If the Charcot midfoot bones are unstable (Eichenholtz stage 1 or 2), then they can be excised with a wedge excision. The bones on either side of the resection are then fused to recreate the normal arch of the foot and held in place with an Ilizarov frame. The shortening of the skeletal midfoot usually leaves enough loose soft tissue to close the wound primarily or with a local flap.

Hindfoot Coverage

Plantar heel defects or ulcers are among the most difficult of all wounds to heal. If they are the result of the patient being in a prolonged decubitus position, they are also usually a reflection of severe vascular disease. A partial calcanectomy may be required to develop enough of a local soft tissue envelope to cover the resulting defect. Although patients can ambulate with a partially resected calcaneus, they will need orthotics and molded shoes. If there is an underlying collapsed bone or bone spur causing a hindfoot defect, the bone should be shaved down. These ulcers are usually closed with double V–Y flaps or larger medially based rotation flaps. Plantar heel defects can also be closed with pedicled flaps that include the medial plantar fasciocutaneous flap or the Flexor Digiti Minimi muscle flap. Posterior heel defects are better closed with extended lateral calcaneal fasciocutaneous flap or the retrograde sural artery fasciocutaneous flap. If the defect is large, then a free flap should be used (Fig. 21.34). The flap should be carefully tailored so there is no excess tissue and it blends in well with the rest of the heel. Medial or lateral calcaneal defects usually occur after fracture and attempted repair. If this results in osteomyelitis of the calcaneus, the infected bone should be debrided and antibiotic beads should be placed. The defect can usually be covered with the Abductor Hallucis muscle flap medially or the Abductor Digiti Minimi flap laterally. The exposed muscle is then skin grafted. After 6 or more weeks, the beads can be replaced with bone graft. Consideration should be given to applying an Ilizarov frame during the healing phase for heel defect because it protects the soft tissue repair from pressure by suspending the heel and immobilizes the ankle so that sheer forces cannot disrupt the repair.

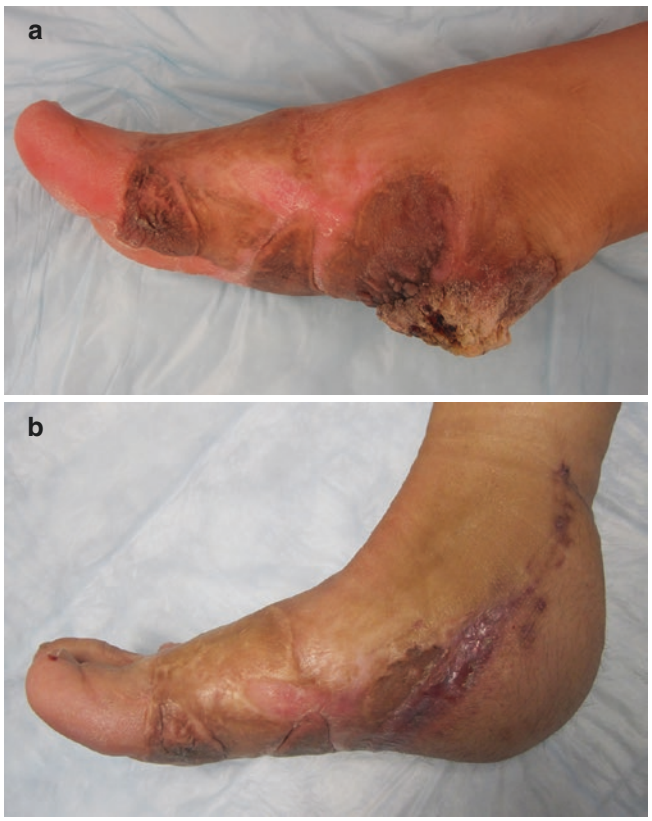

Fig. 21.34 Chronic calcaneal wound with verrucous carcinoma transformation (**a**). Hindfoot reconstruction with ALT flap neurotized to medial calcaneal nerve (**b**), so that she could fit into normal shoe gear and ambulate with sensory feedback

The two hindfoot amputations are the Chopart and Symes amputations. The Chopart amputation leaves an intact talus and calcaneus while removing the mid and forefoot bones of the foot. To avoid going into equino-varus deformity, a minimum of two centimeters of the Achilles has to be resected so that the connection between the two parts of the Achilles tendon have no chance of healing together. When the amputation has healed, a calcaneal-tibial rod can be used to further stabilize the position of the calcaneus. The Symes amputation should be considered if there is insufficient tissue to primarily close a Chopart amputation and the talus and calcaneus are involved with osteomyelitis. The tibia and fibula are cut just above the ankle mortise and the de-boned heel pad swung anteriorly. The heel pad has to be anchored to the anterior portion of the distal tibia to prevent posterior migration. The ultimate goal is a thin, tailored stump that can fit well into a patellar weight-bearing prosthesis. A poorly designed Symes amputation is a prosthetist's nightmare and can lead to repeated breakdown of the stump.

Dorsum of the Foot

The defects on the dorsum of the foot are often treated with simple skin grafts. If the tissue covering the extensor tendons is thin or nonexistent, a dermal regeneration template can be applied. When the dermis is vascularized, a thin skin autograft is then applied (Fig. 21.35). Local flaps that can be used for small defects include rotation, bilobed, rhomboid, or transposition flaps. Possible pedicled flaps include the Extensor Digitorum Brevis (EDB) muscle flap, the dorsalis pedis flap, the supra-malleolar flap, and the sural artery flap. The EDB muscle's reach can be increased by cutting the dorsalis pedis artery above or below the lateral tarsal artery, depending on where the defect is and whether there is adequate antegrade and retrograde flow. The reach of the supra-malleolar flap can be increased by cutting the anterior perforating branch of the peroneal artery before it anastomoses with the lateral malleolar artery. For defects at the sinus tarsus the EDB flap works well. The most appropriate microsurgical free flap is a thin fasciocutaneous flap to minimize bulk. The radial forearm flap is a good choice because it is sensate and provides a vascularized tendon (palmaris tendon) to reconstruct lost extensor function. Thin muscle flaps with skin grafts or fascial flaps are effective options as well.

Ankle Defects

Soft tissue around the ankle is sparse and has minimal flexibility. If there is sufficient granulation tissue, a skin graft will work well. To encourage the formation of a healthy wound bed, NPWT with or without Integra can be used. The Achilles tendon, if allowed sufficient time to form a granulating bed, will tolerate a skin graft that will hold up well over time. Local flaps do not need to cover the entire defect because only the critical area of the wound such as exposed tendon, bone, or joints needs to be covered while the rest of the wound can be skin grafted. Useful local flaps include rotation, bilobed, or transposition flaps. Local flaps can easily be individually designed off posterior tibial and peroneal arterial perforators. Pedicled flaps include the supra-malleolar flap, the dorsalis pedis flap, the retrograde sural artery flap, the medial plantar flap, Abductor Hallucis muscle flap, the Abductor Digiti Minimi muscle flap, and the Extensor Digitorum Brevis muscle flap. Free flaps can either be fasciocutaneous or muscle with skin graft but they should be kept thin. The medial sural perforator flap can be an excellent choice, as it provides thin tissue, without the harvest of a major artery or muscle (Fig. 21.36). Additionally, the wound can be kept on the ipsilateral leg. In order to ensure good healing, the ankle should be temporarily immobilized with an external fixator.

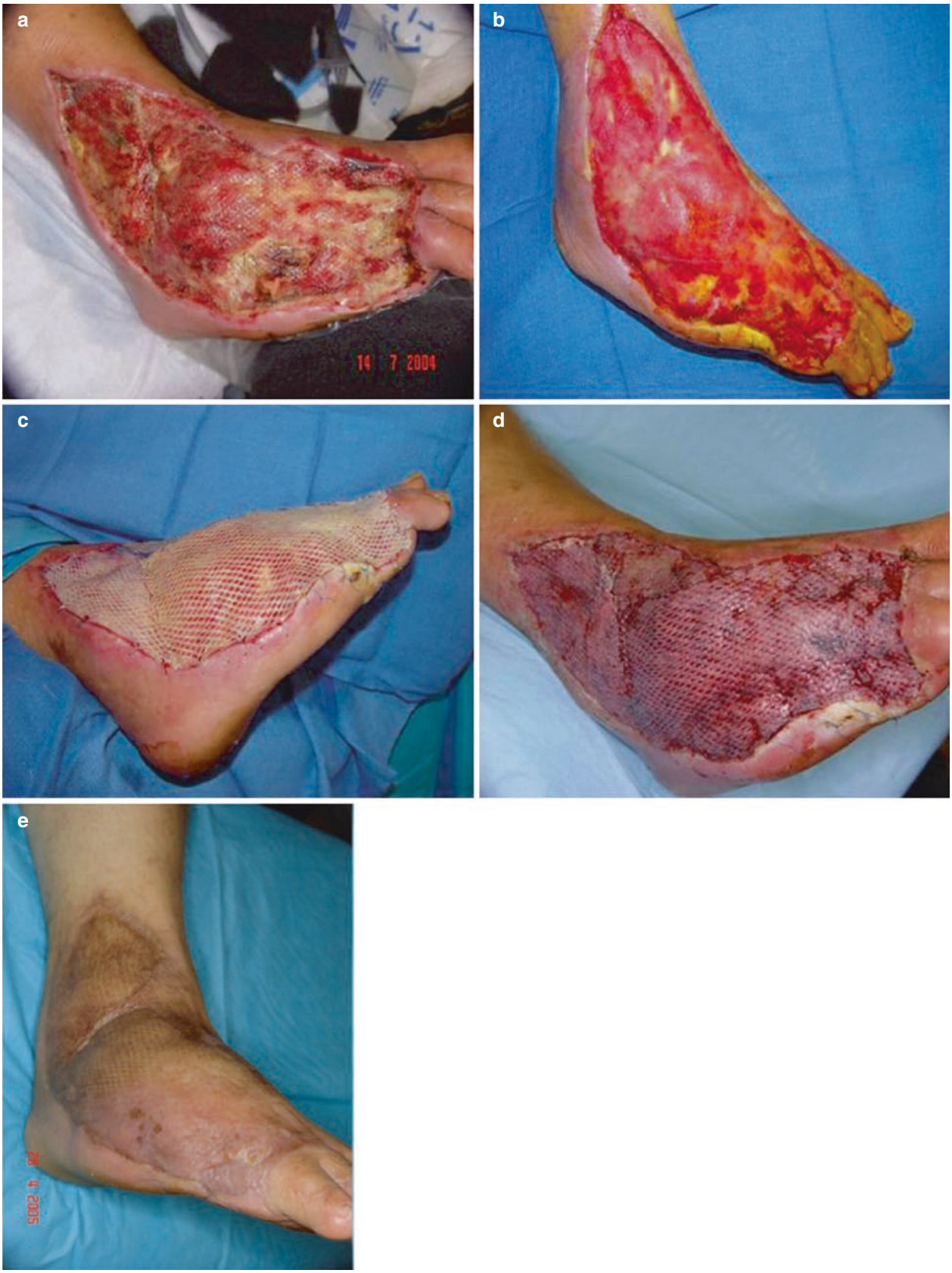

Fig. 21.35 This patient developed necrotizing fasciitis with alpha-hemolytic streptococcus that destroyed the entire dorsum of the foot (**a**). After multiple debridements, the wound was covered with neodermis and NPWT. Neodermis (**b**) was then covered with a skin graft (**c**) and the wound went on to heal without incident (**d**, **e**)

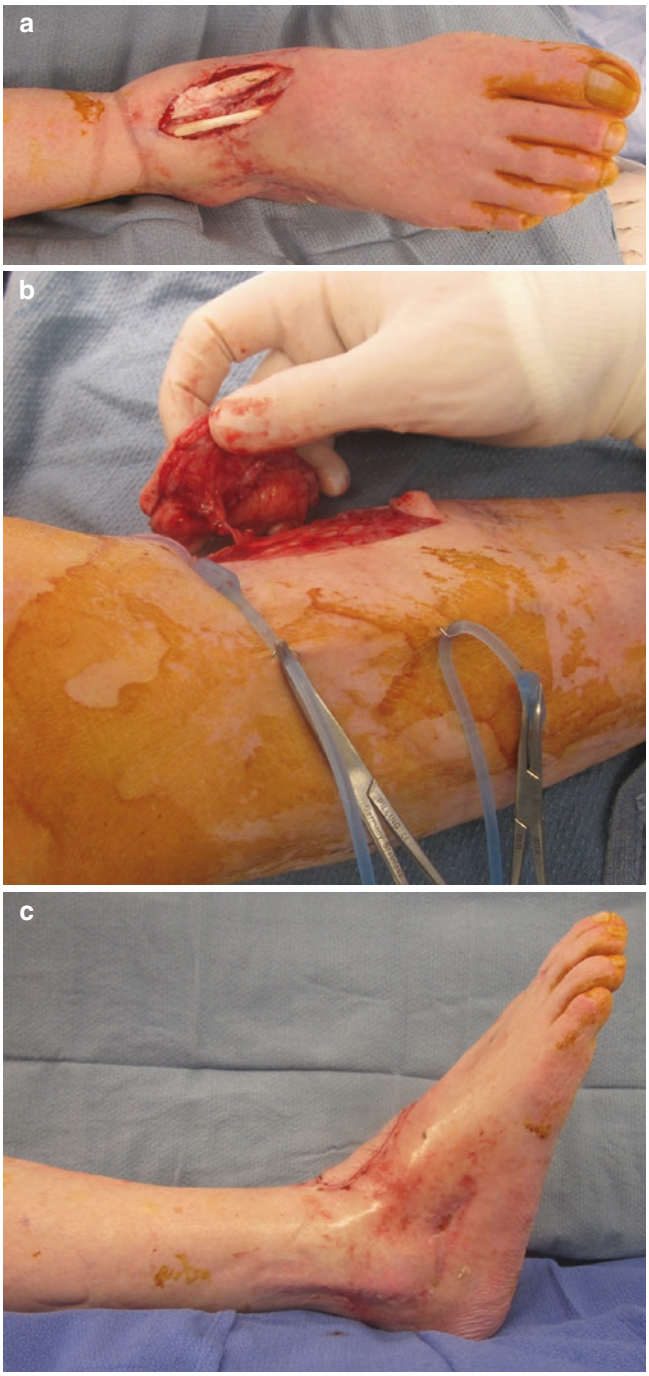

Fig. 21.36 Chronic dorsal foot wound debrided to healthy tissue, with exposed but vitalized tibialis anterior and extensor hallucis longus tendon (**a**). A thin, medial sural perforator flap from the ipsilateral leg was utilized for tendon resurfacing. The anastomosis was performed in an end-to-side fashion with the dorsalis pedis artery (**b**, **c**)

Summary

Treating diabetic foot ulcers and gangrene can only be done effectively by using a team approach which at the minimum includes a wound care team, a vascular surgeon, a foot and ankle surgeon, a plastic surgeon, an infectious disease specialist, an endocrinologist, and a prosthetist. The reconstruction is dictated by how much of the foot remains after adequate debridement and how the foot can be closed in the most biomechanically stable construct possible. This may involve skeletal manipulation, tendon lengthening, and or partial foot amputations. Soft tissue reconstruction can be as simple as allowing the wound to heal by secondary intention or as complex as microsurgical free flaps. Wound healing adjuncts such as growth factors, cultured skin, and hyperbaric oxygen are helpful adjuncts. Over 90% of the wound can be closed utilizing simple methods from healing by secondary intention to skin grafting. Utilizing this approach should decrease the primary and secondary major amputation rate to below 5%.

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The Diabetic Charcot Foot

22

Lee C. Rogers and Robert G. Frykberg

Abstract

The diabetic Charcot foot is a potentially limb-threatening deformity associated with peripheral neuropathy and concomitant injury. Often the precipitating injury is fairly minor, but unrecognized due to the underlying peripheral sensory neuropathy. With existing loss of protective sensation the neuropathic individual continues to walk on the injured extremity causing progressive inflammation with varying degrees of bone and joint pathology. Severe deformity can ensue that predisposes to ulceration, infection, and potential amputation. It is therefore critical to diagnose this condition early in its natural history to prevent progressive foot or ankle deformity and instability.

This chapter reviews the etiology, diagnostic methods, and various treatment options for the active and inactive Charcot arthropathy of the foot and ankle.

Introduction

The Charcot foot is a devastating but oftentimes preventable complication of diabetes with peripheral neuropathy. The condition has several synonyms including Charcot's arthropathy, Charcot joint disease, Charcot syndrome, neuroarthropathy, osteoarthropathy, and many derivations or combinations thereof. It is named after Jean-Martin Charcot (1825–1893), a French neurologist who first described the joint disease associated with tabes dorsalis and named it the "arthropathy of locomotor ataxia." In 1881, J.-M. Charcot presented his findings at the 7th International Medical Congress in London which was attended by many acclaimed physicians of the era. During this meeting the eponym "Charcot's Disease" was designated by Sir James Paget to these degenerative neuropathic changes in bones and joints [1, 2]. Although W. Musgrave in 1703 and later J.K. Mitchell in 1831 ostensibly described osteoarthropathy associated with venereal disease and spinal cord lesions, respectively, Charcot's name remains synonymous with neuropathic arthropathies regardless of etiology [3].

W.R. Jordan in 1936 was the first to fully recognize and report on the association of neuropathic arthropathy with diabetes mellitus [4, 5]. In that comprehensive review of the neuritic manifestations of diabetes, he described a 56-year-old woman with diabetes duration of approximately 14 years who presented with "a rather typical, painless Charcot joint of the ankle." His description typifies the classic presentation we now commonly recognize in patients with long-standing diabetes and neuropathy. Subsequently, Bailey and Root in their 1947 series noted that 1 in 1100 patients with diabetes mellitus developed neurogenic osteoarthropathy [5]. In the classic 1972 Joslin Clinic review of 68,000 patients by Sinha et al., 101 patients were encountered with diabetic Charcot feet [6]. This ratio of 1 case in 680 patients with diabetes brought greater attention to this disorder and characterized the affected patients' clinical and radiographic presentations. In the subsequent 30 years there has been a significant increase in the number of reports on diabetic neuroarthropathy, its complications, and management [4–8]. The prevalence of this condition is highly variable, ranging from 0.15% of all diabetic patients to as high as 29% in a population of only neuropathic diabetic subjects [2, 6, 8, 9]. A prospective study of a large group of patients with diabetes from Texas reported an incidence of 8.5 per thousand per year. Neuroarthropathy was significantly more common in Caucasians than in Mexican Americans (11.7/1000 vs. 6.4/1000) [10]. While this study may give us better insight into the true frequency of neuroarthropathy in diabetes, much of the data we currently rely upon is based upon retrospective studies of small single center cohorts. Nonetheless, the incidence of Charcot foot cases reported is very likely an

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underestimation since many cases go undetected, especially in the early states and cases that receive early appropriate treatment may never be formally diagnosed if the natural history is interrupted [2, 7, 9]. The frequency of diagnosis of the diabetic Charcot foot appears to be increasing as a result of increased awareness of its signs and symptoms [11]. Although the original descriptions of neuropathic osteoarthropathy were attributed to patients with tertiary syphilis, diabetes mellitus has now become the disease most often associated with this severe foot disorder. Not only are patients with Charcot foot deformities at greater risk of amputation than those with neuropathic ulcers but without Charcot foot, a study from the UK has also found them to have a higher mortality [12, 13]. While the power of this study did not allow for significant differences to emerge, it does confirm the need for larger population-based studies to fully elucidate the epidemiology of this limb-threatening complication. Overall, the 4- or 5-year relative mortality rate is 28–45% in those with Charcot foot and diabetes [12, 13]. van Baal reported the life expectancy of someone diagnosed with Charcot foot is 7.9 years in the UK [14].

Etiology and Pathogenesis

Charcot foot can be defined as a noninfectious and progressive condition of single or multiple joints characterized by joint dislocation, pathologic fractures, and severe destruction of the pedal architecture which is closely associated with peripheral neuropathy [2, 7]. Almost uniformly, trauma of some degree when superimposed on the neuropathic extremity precipitates the cascade of events leading to the joint destruction. Neuroarthropathy, therefore, may result in debilitating deformity with subsequent ulceration and even amputation [15, 16]. Charcot foot can result from various disorders which have the potential to cause a peripheral neuropathy. With the decline in numbers of patients with tertiary syphilis since Charcot's time and the concomitant rise in prevalence of diabetes mellitus, the latter disease has now become the primary condition associated with the Charcot foot.

There are several conditions producing radiographic changes similar to Charcot joints. These include acute arthritides, psoriatic arthritis, osteoarthritis, osteomyelitis, osseous tumors, and gout. These joint affectations, in the presence of neuropathy, make the correct diagnosis even more difficult to ascertain [6]. Nonetheless, the characteristics of the joint changes, site for predilection, and clinical correlation assist in determining the true underlying diagnosis.

The primary risk factors for this potentially limb-threatening deformity are the presence of dense peripheral neuropathy, normal circulation, and a history of preceding trauma, often minor in nature and may be unnoticed [15, 17]. There is no apparent predilection for either sex [2]. Trauma is not necessarily limited to typical injuries such as sprains, contusions, or fractures. Foot deformities, prior amputations, and joint infections may result in sufficient stress that can lead to neuroathropathy. Likewise, foot surgery in a patient with neuropathy can result in enough trauma and spark a Charcot event [18]. Renal and/or pancreatic transplantation have also been implicated as an inciting event leading to the development of a Charcot foot [19, 20].

Although the exact pathogenesis may vary from patient to patient, it is undoubtedly multifactorial in nature [17, 21]. The *neurotraumatic* (German) theory has traditionally been proposed as the primary etiology of osteoarthropathy in which neuropathy and repeated trauma produce eventual joint destruction. The loss or diminution of protective sensation allows repetitive micro- or macrotrauma producing intracapsular effusions, ligamentous laxity, and joint instability. With continued use of the injured extremity further degeneration ensues that eventually results in a Charcot joint. Underlying sensory neuropathy resulting from any disorder is therefore a prerequisite under this theory of pathogenesis. However, the neurotraumatic theory does not explain all accounts of Charcot arthropathy, especially its occurrence in bedridden patients [2, 7, 15].

The *neurovascular reflex* (French) theory, in contrast, proposes that increased peripheral blood flow due to autonomic neuropathy leads to hyperemic bone resorption [22]. This theory might indeed correspond to Charcot's original hypothesis of a central "nutritional" defect, although we now recognize this process as a *peripheral* nerve disorder. Autonomic neuropathy (and endothelial dysfunction) results in an impairment of vascular smooth muscle tone and consequently produces a vasodilatory condition in the small arteries of the distal extremities [23, 24]. Impairment of neurogenic vascular responses in patients with diabetic neuropathy has been supported by one study that consequently also showed preserved maximal hyperemic responses to skin heating in patients with Charcot arthropathy [23, 25]. In concert with associated arteriovenous shunting there is a demonstrable increase in bone blood flow in the neuropathic limb. The resultant osteolysis, demineralization, and weakening of bone can predispose to the development of Charcot foot [2, 17, 22, 25–27]. Several studies have demonstrated reduced bone mineral density with an apparent imbalance between the normally linked bone resorption and production in patients with osteoarthropathy [27–29]. Specifically, greater osteoclastic than osteoblastic activity has been noted in acute neuroarthropathy, suggesting an explanation for the excessive bone resorption during the acutely active stage [23, 27].

The actual pathogenesis of Charcot arthropathy most likely is a combined effect of both the neurovascular and neurotraumatic theories [17, 26, 30]. It is generally accepted that trauma superimposed on a well-perfused, but severely

neuropathic, extremity can precipitate the development of an acute Charcot foot. Approximately 50% of those with Charcot foot recall some incipient trauma [31]. But the presence of sensory neuropathy can render the patient unaware of the initial precipitating trauma and often profound osseous destruction takes place during continued ambulation. The concomitant autonomic neuropathy with its associated osteopenia and relative weakness of the bone predisposes it to fracture [23, 28]. A vicious cycle then ensues where the insensate patient continues to walk on the injured foot, thereby allowing further damage to occur [7]. With added trauma and fractures in the face of an abundant hyperemic response to injury, marked inflammation and edema soon follows. Capsular and ligamentous distension or rupture is also a part of this process and leads to the typical joint subluxations and loss of normal pedal architecture culminating in the classic rocker-bottom Charcot foot. The amount of joint destruction and deformity which results is highly dependent upon the time at which the proper diagnosis is made and when non-weight-bearing immobilization is begun [7]. A simplified cycle of the pathogenesis of Charcot joints is illustrated in Fig. 22.1.

Tightening of the posterior leg muscle complex (equinus) may play a special role in the development of the Charcot midfoot deformity. Achilles tendons of those with Charcot foot are morphologically different than disease-matched controls [32, 33]. The pull of the tendon on the calcaneus increases the forces resulting in subluxation or dislocation at the midfoot joints (Fig. 22.2).

Often it is a fracture, either intra-articular or extra-articular, which initiates the destructive process. This had not been fully appreciated until Johnson presented a series of cases in which diabetic patients developed typical Charcot joints after sustaining neuropathic fractures [34]. Additionally, amputation of the great toe or first ray, often a consequence of infection or gangrene in the diabetic patient, may lead to neuropathic joint changes in the lesser metatarsophalangeal (MTP) joints and tarsometatarsal (TMT) joints. Presumably, this is a stress-related factor secondary to an acquired biomechanical imbalance. Intra-articular infection can also be implicated as an inciting event leading to this endpoint. In effect, almost any inflammatory or destructive process introduced to a neuropathic joint has the potential for creating a Charcot joint. Herbst et al. have recently reported

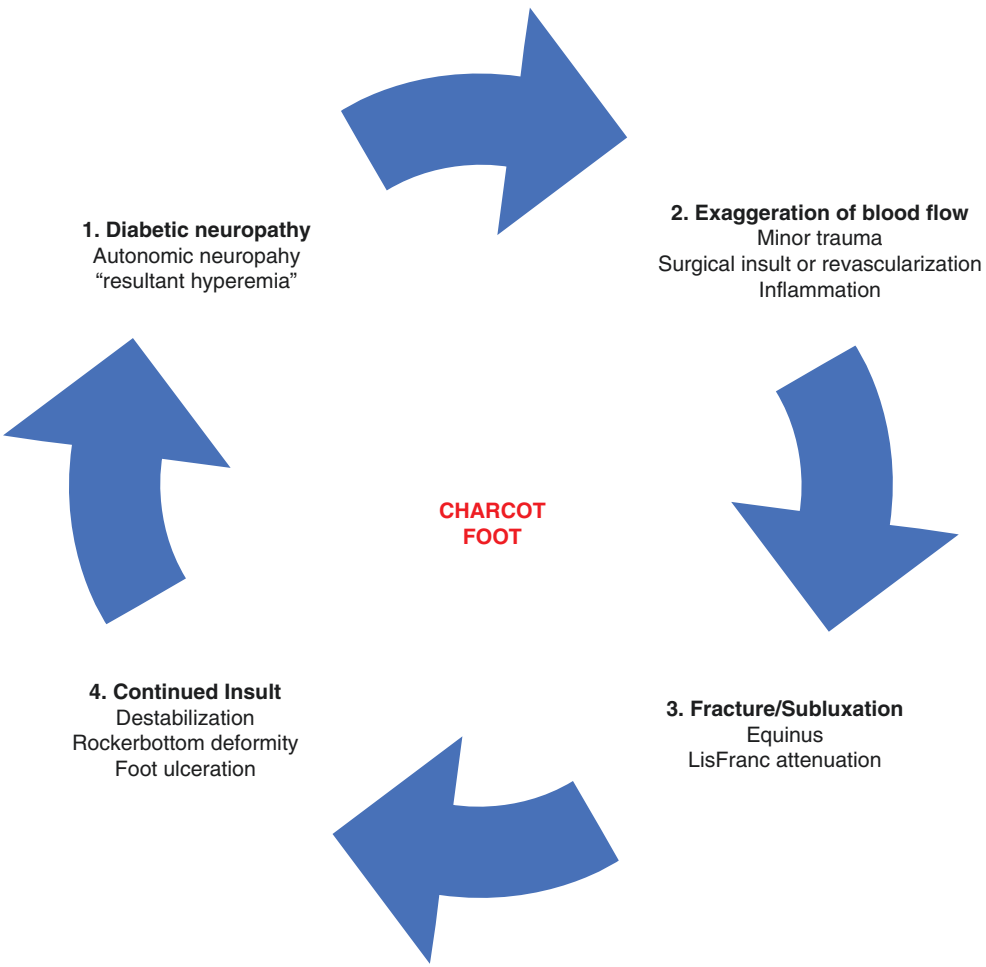

Fig. 22.1 Pathogenic cycle of diabetic neuroarthropathy. (From Lee C. Rogers, with permission)

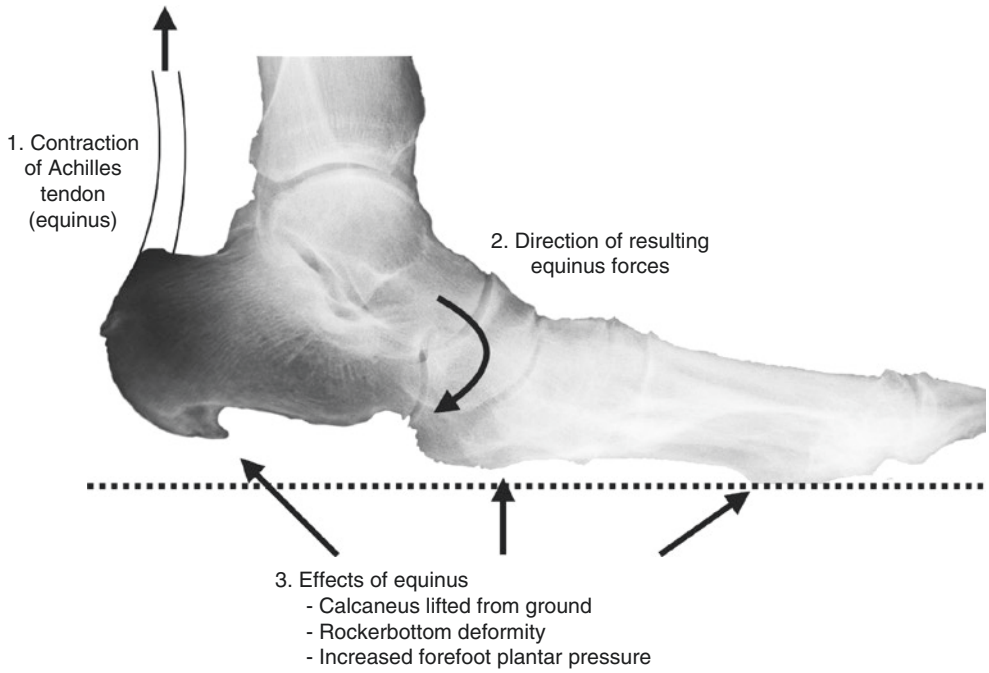

Fig. 22.2 The contribution of the Achilles tendon and equinus to Charcot foot deformity. (From Lee C. Rogers, with permission)

their findings concerning the type of presentation as related to patients' bone mineral density (BMD) [35]. They found that patients with normal BMD had typical changes in the midfoot primarily comprised of joint dislocations. However, in those patients with reduced BMD, fracture patterns predominated in the ankle and forefoot.

Several authors have noted the similarities between the acute destructive phase in Charcot arthropathy and reflex sympathetic dystrophy (complex regional pain syndrome) [23, 24, 36]. Both conditions are associated with an exaggerated vascular response as well as with the development of osteopenia. Both can also be related to previous acute trauma. While the underlying pathophysiological processes are not yet firmly established, both are marked by excessive osteoclastic activity and seem to respond well to treatment with bisphosphonates [36]. Jeffcoate has also suggested that a dysregulation of the RANK-L (receptor activator of nuclear factor kappa B ligand)/OPG (osteoprotegerin) signaling pathway and attendant effects on blood flow and bone turnover might also play a role in this regard [37, 38]. Further study is required, however, to determine how these pathways interact in patients with neuropathy to cause increased vascularity and subsequent osteopenia.

Clinical Presentation

The classic presentation for acute osteoarthropathy includes several characteristic clinical findings which are summarized in Table 22.1. Typically, the patient with a Charcot foot will have had a long duration of diabetes, usually in excess of 12 years. Although all age groups can be affected, a review of the literature in this regard indicates that the majority of patients are in their sixth decade (mid-fifties) [2, 17]. A more recent report, however, indicates that there is an apparent age difference in onset between type 1 and type 2 diabetic patients [39]. Whereas the average age at presentation for the entire cohort and type 2 patients is indeed in the sixth decade, for type 1 patients the age at onset was in the fifth decade (forties). Patients with type 1 diabetes also demonstrated a longer duration of the disease than in type 2 diabetic patients with osteoarthropathy (24 vs. 13 years) [39]. This has also been corroborated by an earlier report from Finland [40]. While unilateral involvement is the most frequent presentation, bilateral Charcot feet can be found in 9–18% of patients [6, 15].

Table 22.1 Clinical features of active Charcot foot

Vascular	Neuropathic	Skeletal	Cutaneous
Bounding pedal pulse	Absent or diminished:	Rocker-bottom deformity	Neuropathic ulcer
Erythema	Pain	Medial tarsal subluxation	Hyperkeratoses
Edema	Vibration	Digital subluxation	Infection
Warmth	Deep tendon reflexes	Rearfoot equinovarus	Gangrene
	Light touch	Hypermobility, crepitus	
	Anhidrosis		

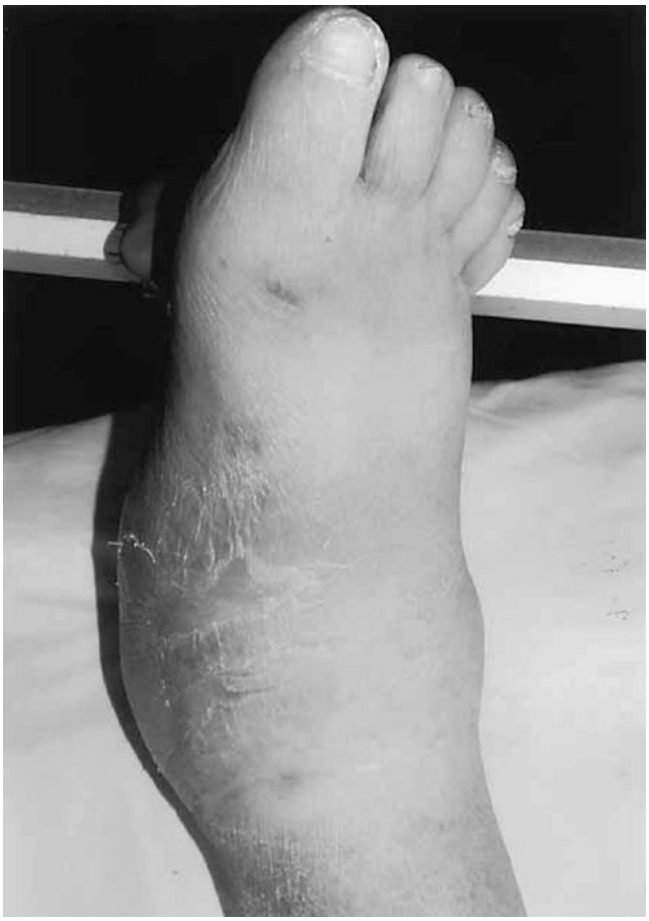

Fig. 22.3 Acute Charcot ankle with profound foot and leg edema

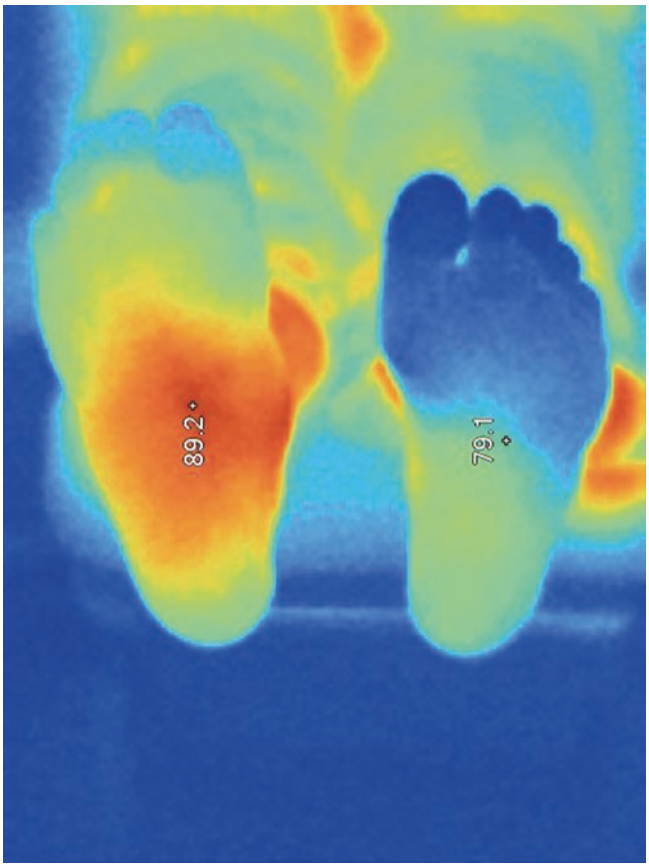

Fig. 22.4 Thermograph of the plantar feet with a significant temperature difference, indicating an active right Charcot foot. (From Lee C. Rogers, with permission)

The initial presentation for acute active Charcot arthropathy is usually quite distinct in that a diabetic patient will seek attention for a profoundly swollen foot that is difficult to fit into a shoe (Fig. 22.3). Although classically described as painless, 75% of these patients will complain of pain or aching in an otherwise insensate foot [15]. Frequently, an antecedent history of some type of injury can be elicited from the patient [31]. When no such history is available, the precipitating event might simply have gone unrecognized in the neuropathic limb.

On examination, the pulses will be characteristically bounding even through the grossly edematous foot [17, 41]. Occasionally, however, the swelling will obscure one or both pedal pulses. In concert with the hyperemic response to injury, the foot will also be somewhat erythematous and warm or hot. The skin temperature elevation can be ascertained by dermal infrared thermometry or thermography and will contrast with the unaffected side by 3–8 °C (Fig. 22.4) [2, 15, 40, 42, 43]. There is always some degree of sensory neuropathy in which reflexes, vibratory sense, proprioception, light touch, and/or pain (pin prick) are either diminished or absent. As mentioned, the patients will most often relate some localized pain although often mild in comparison to the deformity present. Motor neuropathy can present as a foot drop deformity or with intrinsic muscle atrophy. Ankle equinus can sometimes be ascertained initially, but may be difficult to perceive if there is gross osseous deformity and laxity in the midfoot. Autonomic neuropathy, which coexists with somatosensory neuropathy, can be clinically appreciated by the presence of anhidrosis with very dry skin and/or thick callus or by measuring heart rate variability with deep breathing [23, 24]. Another fairly frequent cutaneous finding is a plantar neuropathic ulceration, especially in an active Charcot foot of long duration. A concomitant ulceration will therefore raise questions of potential contiguous osteomyelitis [17, 30, 41].

The skeletal changes frequently manifest as obvious deformity of the medial midfoot with collapse of the arch and/or rocker-bottom deformity (Fig. 22.5) [2, 30]. Associated findings might often include hypermobility with crepitus, significant instability, and ankle deformity.

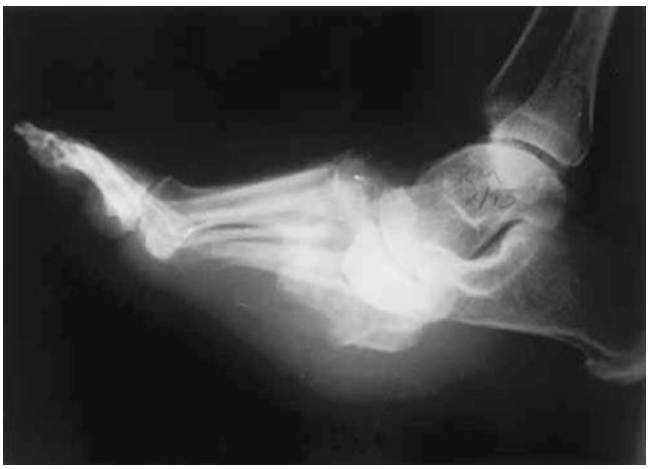

Fig. 22.5 Radiograph of rocker-bottom Charcot foot with collapse of the midfoot

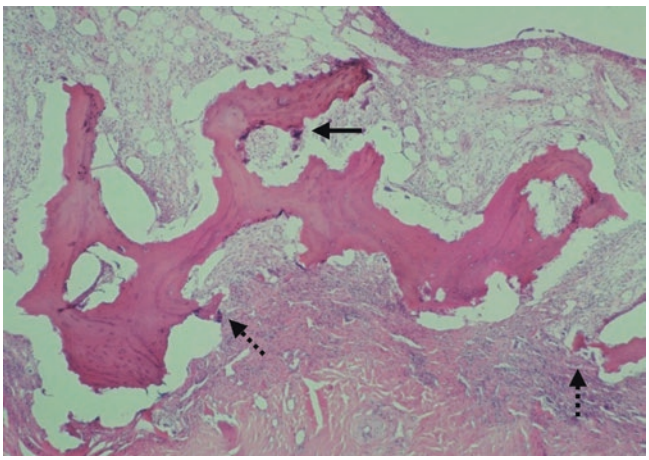

Fig. 22.6 Light micrograph of a pathology slide of bone from a foot with active neuroarthropathy (100×, decalcified, H&E stain). Note the center trabeculum has incongruous edges with osteoclasts (solid arrow), many inflammatory cells, and trabecular fragmentation (broken arrows). (From Lee C. Rogers, with permission)

Diagnosis of Active Charcot Foot

The diagnosis of active Charcot foot is primarily based on history and clinical findings, but should be confirmed with imaging. Inflammation plays a key role in the pathophysiology and is the earliest exam finding [44]. When presented with a warm, swollen, insensate foot, plain radiographs are invaluable in ascertaining the presence of osteoarthropathy [17, 45]. In most cases, no further imaging studies will be required to make the correct diagnosis. However, in the active, prodromal "stage 0" there may be primarily soft tissue changes noted without evidence of distinct bone or joint pathology [46, 47]. Further investigation with scintigraphy, MRI, or serial radiographs should be considered when suspicion is high for osteoarthropathy [48–50]. With a concomitant wound, it may initially be difficult to differentiate between acute Charcot arthropathy and osteomyelitis solely based on plain radiographs [51]. Additional laboratory studies may prove useful in determining the appropriate diagnosis. Leukocytosis can often suggest acute osteomyelitis; however, this normal response to infection can be blunted in persons with diabetes [51, 52]. While the erythrocyte sedimentation rate (ESR) may also be elevated in the case of acute infection, it often responds similarly to any inflammatory process and is therefore nonspecific. When the ulcer probes to bone, a bone biopsy may be helpful in distinguishing between osteomyelitis and osteoarthropathy [17]. A biopsy consisting of multiple shards of bone and soft tissue embedded in the deep layers of synovium is pathognomonic for neuroarthropathy (Fig. 22.6) [53].

Radiographic Imaging

Radiographically, osteoarthropathy takes on the appearance of a severely destructive form of degenerative arthritis. Serial X-rays will customarily demonstrate multiple changes occurring throughout the process and can assist in monitoring disease activity. Rarely will nucleotide scanning, CT, or MRI be necessary to establish the diagnosis. The acute or developmental stage is marked by an abundance of soft tissue edema, osteopenia, multiple fractures, loose bodies, dislocations, or subluxations [30, 54]. These radiographic findings are fairly typical of noninfective bone changes associated with diabetes and have been described well by Newman [55]. In addition to alterations in the normal pedal architecture, the metatarsal heads and phalanges will frequently demonstrate atrophic changes often called diabetic *osteolysis*. Synonyms for this phenomenon include a "sucked candy" appearance, "pencil pointing," "hour glass" deformities of the phalanges, or mortar and pestle deformity of the MTP joints. Massive osteolysis can also occur in the rearfoot during the acute stage, especially in the ankle and subtalar joints (Fig. 22.7). These

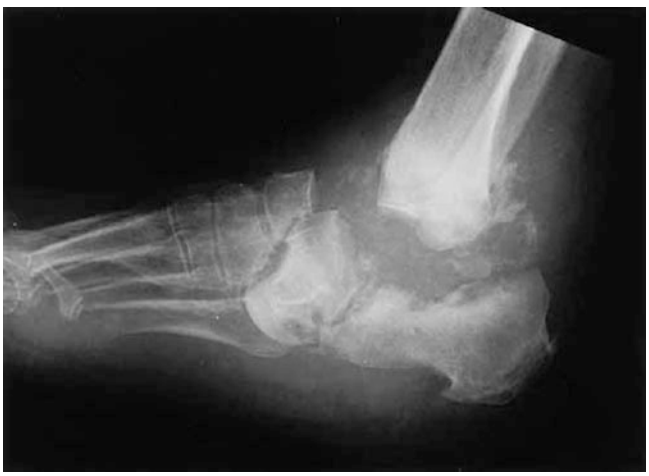

Fig. 22.7 Osteolysis of the talus and disintegration of the ankle and Subtalar joints

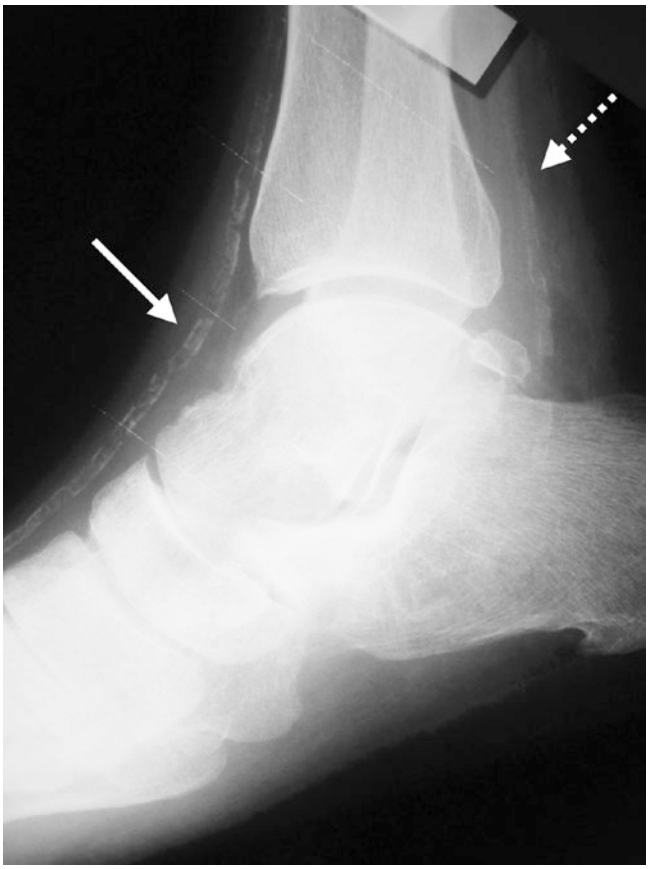

Fig. 22.8 Calcification of the vascular intima media (Monckeberg's sclerosis) can be seen in many patients with Charcot foot. In this lateral ankle radiograph the anterior tibial/dorsalis pedis (solid arrow) and the posterior tibial (broken arrow) arteries are visible. (From Lee C. Rogers, with permission)

changes will often coexist with the obvious fractures that initiated the destructive process. Medial arterial calcification is another associated finding in Charcot arthropathy (Fig. 22.8) [23].

Chronic reparative or quiescent radiographic changes include hypertrophic changes such as periosteal new bone formation, coalescence of fractures and bony fragments, sclerosis, remineralization, and a reduction in soft tissue edema [2, 17, 53]. Rocker-bottom deformities, calcaneal equinus, dropped cuboid, or other deformities not previously appreciated may also become visible, especially when taking weight-bearing images. Lateral weight-bearing foot radiographs are invaluable since they show two important radiographic features of Charcot foot deformities, the calcaneal inclination angle and the talo-first metatarsal relationship. The calcaneal inclination angle (normally 20°) is often reduced or in declination (negative angle). The lateral talo-first metatarsal relationship (a line bisecting the talus and the first metatarsal) should be unbroken (Fig. 22.9). Table 22.2 summarizes the varieties of radiographic changes found in neuroarthropathy.

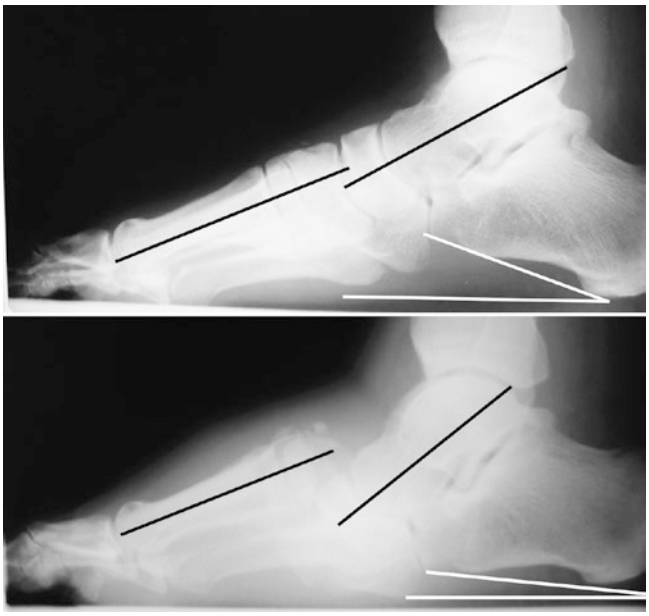

Fig. 22.9 Lateral weight-bearing radiograph of the foot in the same patient before (top) and after (bottom) the development of a rocker-bottom deformity. The calcaneal inclination angle (white line) has decreased and the talo-first metatarsal relationship (black line) is broken in the bottom image. (From Lee C. Rogers, with permission)

Table 22.2 Radiographic changes in neuroarthropathy

Stage	Atrophic changes	Hypertrophic changes	Miscellaneous
Active	Osteolysis – Resorption of bone	Periosteal new bone Intra-articular debris, Joint mice, fragments	Joint effusions Subluxations Fractures
	Metatarsal heads, Phalangeal diaphyses, MTP, subtalar, ankle	Osteophytes, Architectural collapse, Deformity	Soft tissue edema Medial arterial calcification Ulceration
	Osteopenia		
Inactive	Distal metatarsal and rearfoot osteolysis, Bone loss	Periosteal new bone, Marginal osteophytes, Fracture bone callus	Resorption of debris Diminished edema Sclerosis
		Rocker bottom, Midfoot or ankle deformity	Ulceration
		Ankylosis	

Sanders and Frykberg described radiographic patterns of joint involvement based upon joint location in diabetic patients [2]. These patterns may exist independently or in combination with each other as determined through clinical and radiographic findings. They are illustrated in Fig. 22.10 and described as follows: Pattern

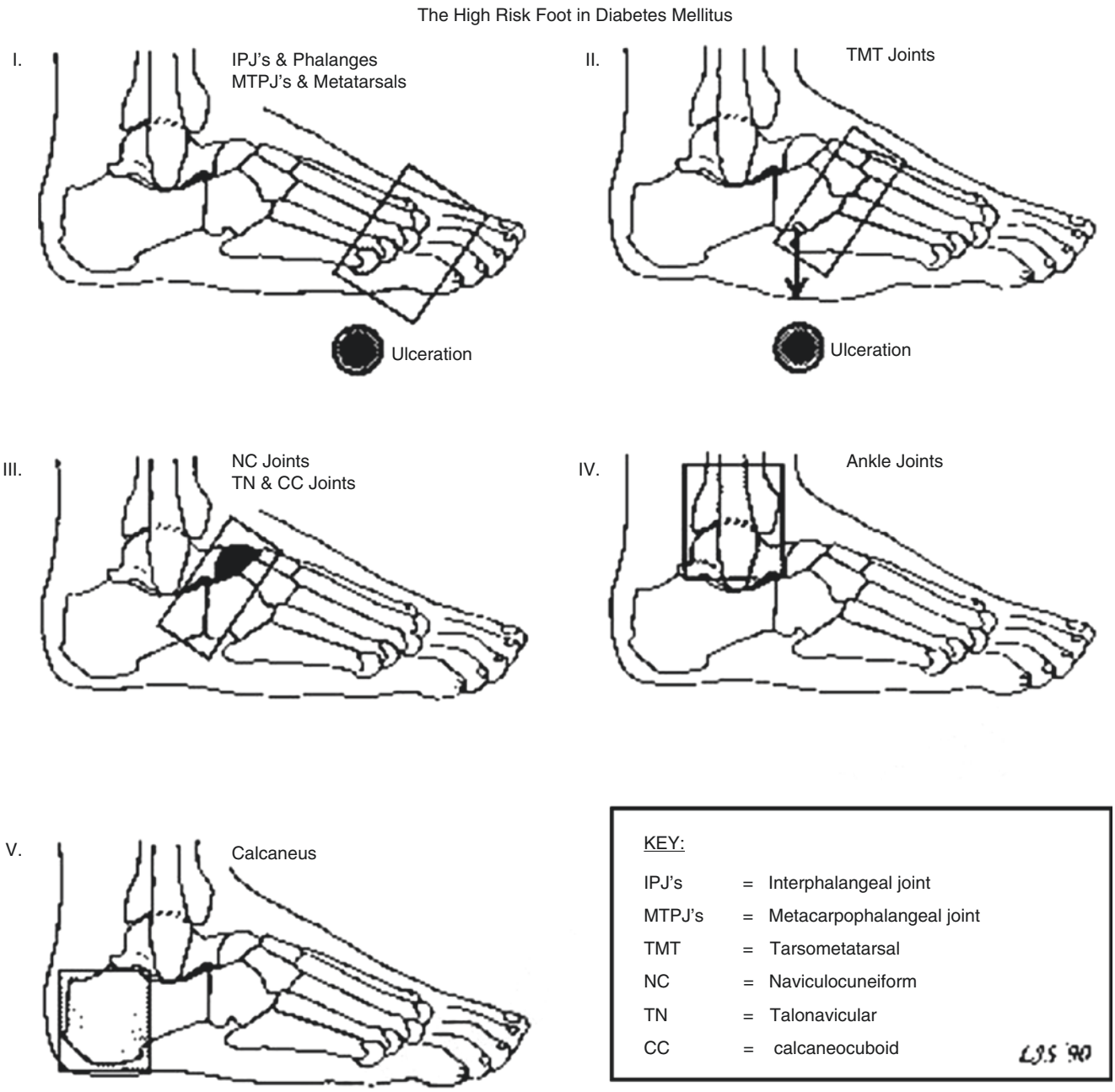

Fig. 22.10 Patterns of diabetic osteoarthropathy based on anatomic sites of involvements. (from Sanders LJ, Frykberg RG. The Charcot foot. In: Frykberg RG, editor. The high risk foot in diabetes mellitus. New York: Churchill Linvingston; 1991. p. 325–35,with permission)

I—Forefoot—Metatarsal-phalangeal joints, Pattern II—Tarsometatarsal (Lisfranc's) joint, Pattern III—Midtarsal and navicular-cuneiform joints, Pattern IV—Ankle and subtalar joints, and Pattern V—Calcaneus (Calcaneal Insufficiency Avulsion Fracture) [2, 29, 30].

Pattern I: Forefoot

Pattern I encompasses atrophic changes or osteolysis of the metatarsophalangeal and interphalangeal joints with the characteristic sucked candy appearance of the distal metatarsals

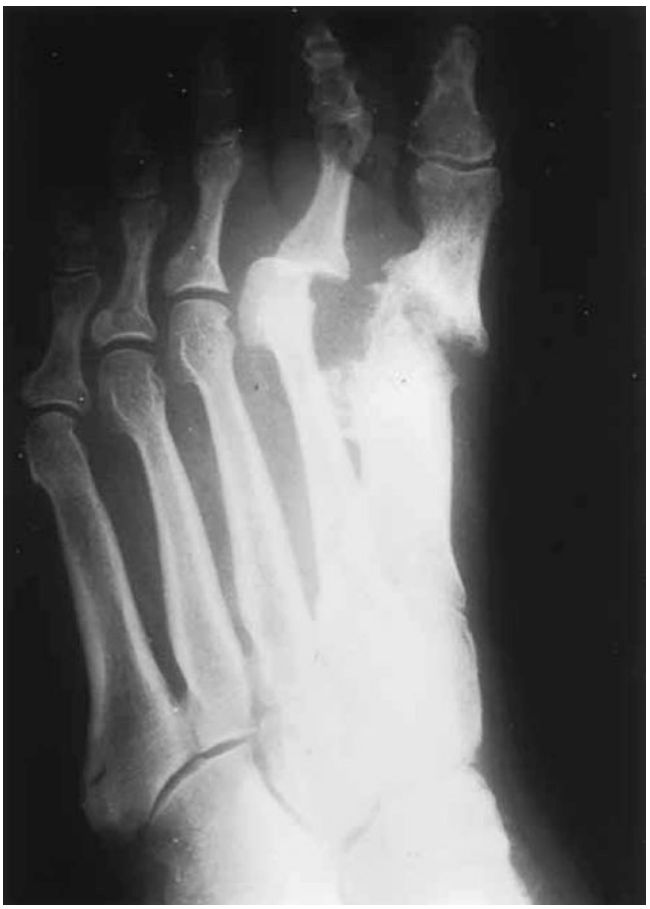

Fig. 22.11 Pattern I: osteolytic changes involving the first metatarsals and phalanx are evident without any current infection documented

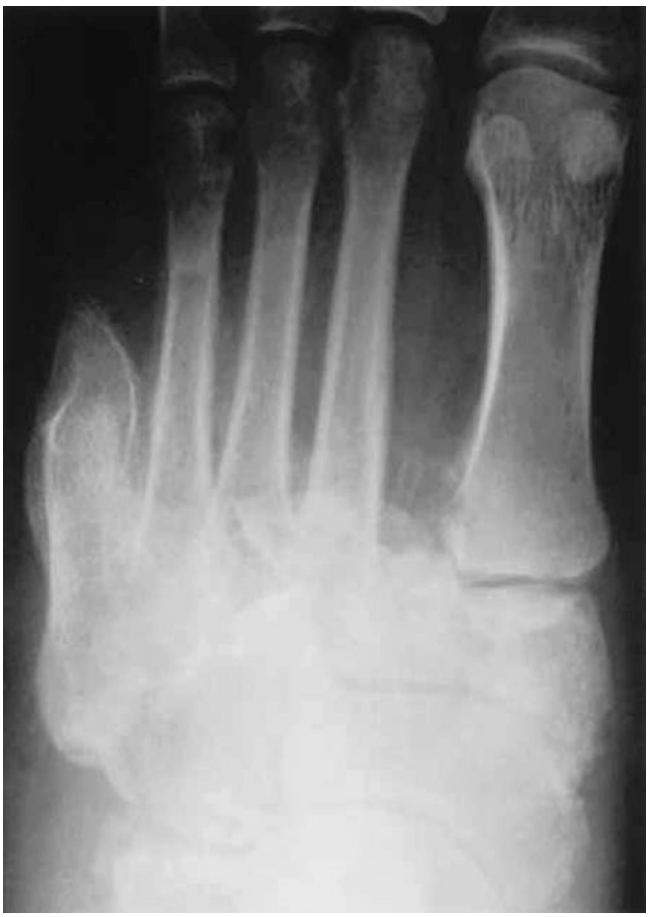

Fig. 22.12 Pattern II: Lisfranc's joint dislocation with associated fractures is evident in this common presentation of the Charcot foot. (Fifth ray had previously been amputated)

(Fig. 22.11) [41]. Frequently, atrophic bone resorption of the distal metatarsals and phalanges accompanies other changes found in the midfoot and rearfoot. An infectious etiology has been proposed for these findings although osteolysis can occur without any prior history of joint sepsis. Reports of 10–30% of the neuroarthropathies have been categorized as Pattern I [6, 22].

Pattern II: Tarsometatarsal (Lisfranc's) Joint

Pattern II involves Lisfranc's joint, typically with the earliest clue being a very subtle lateral deviation of the base of the second metatarsal at the cuneiform joint. Once the stability of this "keystone" is lost, the Lisfranc's joint complex will often subluxate dorsolaterally.

Fracture of the second metatarsal base allows for greater mobility in which subluxation of the metatarsal bases will occur. The rupture of intermetatarsal and tarsometatarsal ligaments plantarly will also allow a collapse of the arch during normal weight-bearing, leading to the classic rocker-bottom deformity. Compensatory contracture of the gastrocnemius muscle will frequently follow and create a further plantarflexory moment to accentuate the inverted arch. This pattern also is commonly associated with plantar ulcerations at the apex of the collapse, which typically involves the cuboid or cuneiforms [2, 17]. This was the most frequent pattern of presentation for diabetic Charcot feet in the Sinha series and represents the most common presentation in clinical practice (Fig. 22.12) [6].

Pattern III: Midtarsal and Naviculocuneiform Joints

Pattern III incorporates changes within the midtarsal (Chopart's) joint with the frequent addition of the naviculocuneiform joint. As described by Newman [55] and Lesko and Maurer [55, 56], spontaneous dislocation of the talona-

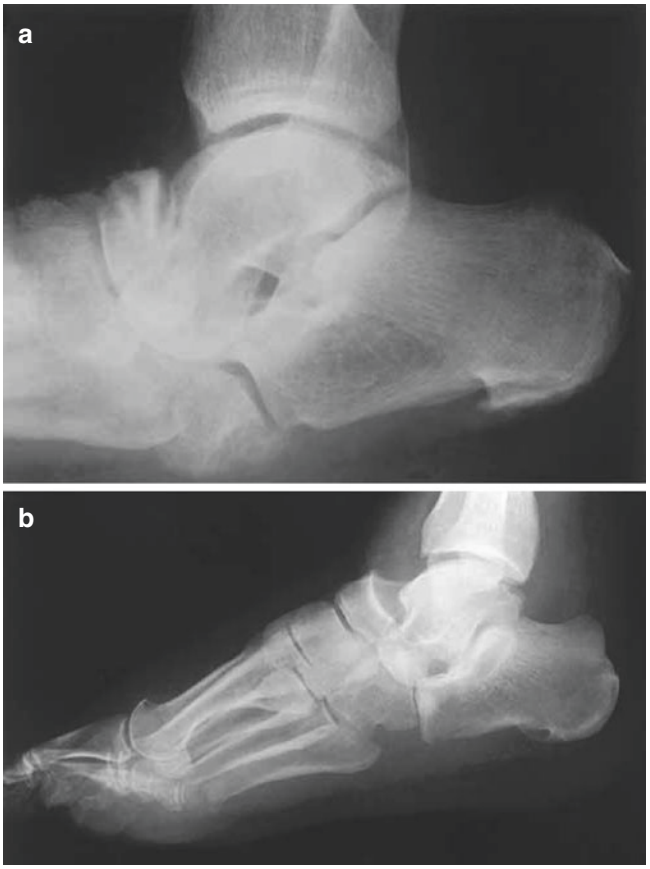

Fig. 22.13 Pattern III: (**a**) Talonavicular dislocation with "dropped cuboid" and plantarflexed calcaneus. (**b**) Talonavicular dislocation with early subtalar and calcaneal-cuboid subluxation. Note absence of fractures or osteochondral defects

vicular joint with or without fragmentation characterizes this pattern. Newman further suggests that isolated talonavicular joint subluxation might even be considered as an entity separate from osteoarthropathy, although still an important element of noninfective neuropathic bone disease [55]. Lisfranc's joint changes (Pattern II) are often seen in combination with Pattern III deformities of the lesser tarsus (Fig. 22.13).

Pattern IV: Ankle and Subtalar Joint

Pattern IV involves the ankle joint, including the subtalar joint and body of the talus (Fig. 22.14). Disintegration of the talar body is equivalent to the central tarsal disintegration of Harris and Brand [57]. The destructive forces are created by joint incongruity and continued mechanical stress which eventually erodes the talus. Massive osteolysis is frequently observed in this pattern with attendant ankle or subtalar subluxation and angular deformity. As noted, tibial or fibular malleolar fractures frequently are seen in association with neuroarthropathy in this location and most likely precipitated

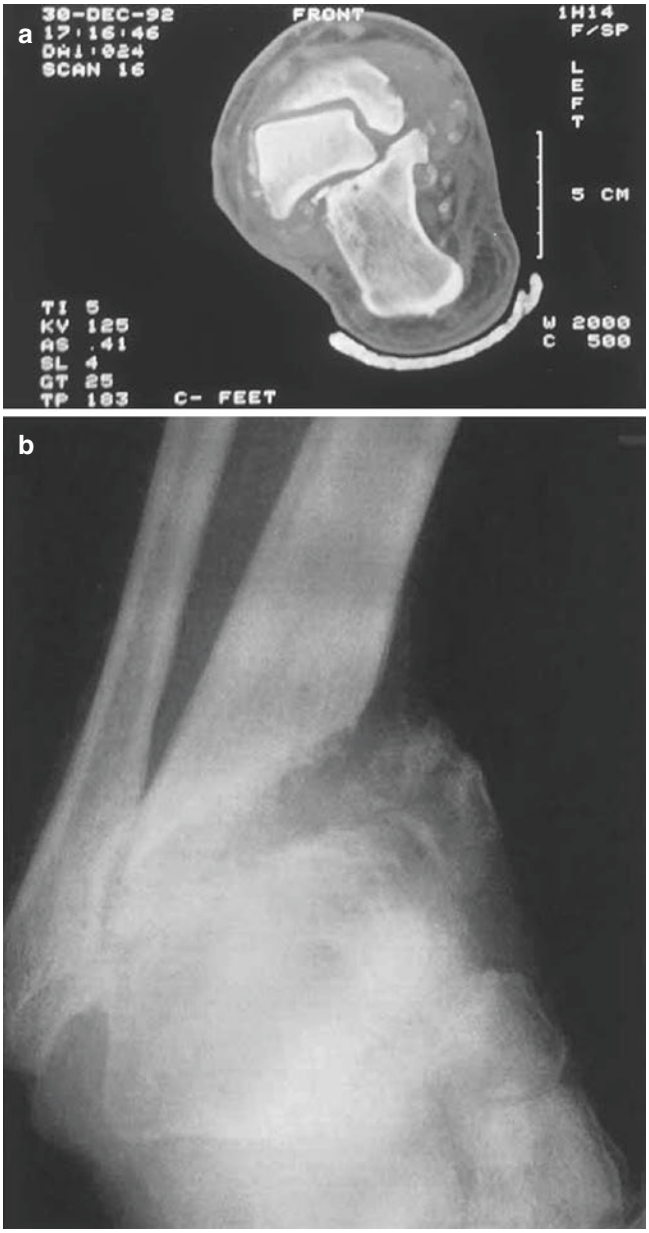

Fig. 22.14 Pattern IV (**a**) Subtalar joint dislocation diagnosed on CT Scan. (**b**) Acute ankle Charcot with medial malleolar fracture and medial displacement of foot

the development of the joint dissolution. Pattern IV Charcot is found in approximately 10% of reported cases [2, 6].

Pattern V: Calcaneus (Calcaneal Insufficiency Avulsion Fracture).

Pattern V, the least common presentation (~2%), is characterized by extra-articular fractures of the calcaneus (posterior pillar). This extra-articular fracture is included in the neuropathic osteoarthropathy classification; however, there is no joint involvement (Fig. 22.15). This is more appropriately

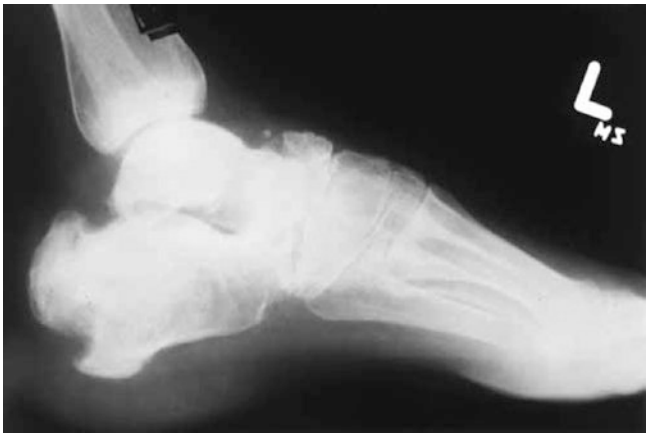

Fig. 22.15 Pattern V: Calcaneal insufficiency avulsion fracture of the calcaneus

considered as a neuropathic fracture of the body or, more commonly, the posterior tuberosity of the calcaneus. El-Khoury and Kathol [58, 59] have termed this entity the "calcaneal insufficiency avulsion fracture."

Advanced Imaging

Technetium (Tc^{99}) bone scans are exquisitely sensitive for detecting Charcot arthropathy but are generally nonspecific in assisting in the differentiation between osteomyelitis and acute neuroarthropathy [48, 60, 61]. Indium (In^{111}) scanning has been shown to be more specific for infection [50, 61–63]. However, false-positive scans can frequently be found in a rapidly evolving acute osteoarthropathy without associated osteomyelitis. Additional studies helpful in differentiating Charcot arthropathy from osteomyelitis include Tc-HMPAO labeled white blood cell scans and magnetic resonance imaging [49, 60, 64, 65].

MRI examination can also be very sensitive to the earliest changes in neuroarthropathy, but again, it is difficult to reliably detect bone infection superimposed upon the gross changes noted surrounding a Charcot joint [49, 51, 60]. Morrison suggests the consideration of "secondary signs" of osteomyelitis may help the clinician discern between Charcot foot and osteomyelitis on MRI [66]. Table 22.3 lists the secondary signs of Charcot foot and osteomyelitis.

Another imaging modality that may show some promise in this regard is positron emission tomography (PET). Hopfner and colleagues recently reported that this modality could not only detect early osteoarthropathy with 95% sensitivity, but could also reliably distinguish between Charcot lesions and osteomyelitis even in the presence of implanted hardware [67]. However, no study is 100% accurate in distinguishing neuropathic bone lesions from infectious entities. Therefore, clinical acumen is necessary for detecting Charcot

Table 22.3 "Secondary signs" of Charcot foot or osteomyelitis on MRI

	Charcot foot	Osteomyelitis
Characteristic	No visible track to bone Primarily affects midfoot	Visible track from skin to bone Primarily affect forefoot and rearfoot
	Multiple bones involved Deformity is common	Usually only one bone affected Deformity is uncommon

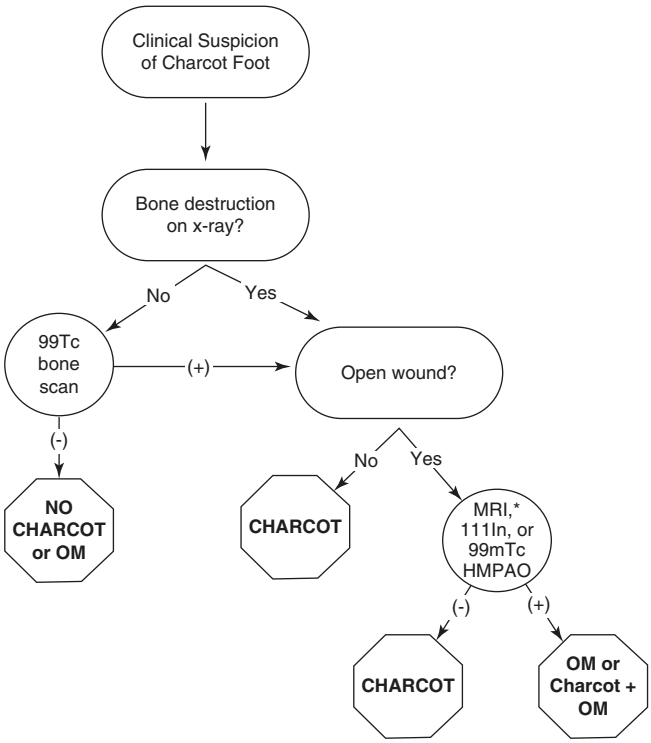

Fig. 22.16 Standard imaging studies to aid in differentiating Charcot foot from osteomyelitis. (From Rogers LC, Bevilacqua NJ. Imaging of the Charcot foot. *Clin Podiatr Med Surg*. 2008;25(2):263–274, vii., with permission)

arthropathy at its onset, and clinical judgment remains of paramount importance in properly assessing and managing these patients. Rogers and Bevilaqua presented a simplified algorithm based on imaging study to help differentiate Charcot foot from osteomyelitis (Fig. 22.16) [51].

Classification of Charcot Arthropathy

The most common classification system of Charcot arthropathy is based on the radiographic appearance as well as physiologic stages of the process. The *Eichenholtz classification* divides osteoarthropathy into developmental, coalescence, and reconstructive stages [53]. Several other authors have subsequently proposed an earlier *Stage 0* that corresponds to

the initial inflammatory period following injury but prior to the development of characteristic bony radiographic changes [46, 68, 69]. This prodromal period might be considered as an "osteoarthropathy in situ" stage. The traditional *developmental* stage is characterized by fractures, debris formation, and fragmentation of cartilage and subchondral bone. This is followed by capsular distension, ligamentous laxity, and varying degrees of subluxation and marked soft tissue swelling. Synovial biopsy at this time will show osseous and cartilaginous debris embedded in a thickened synovium, which is pathognomonic for the disease [53]. The *coalescence* stage is marked by the absorption of much of the fine debris, a reduction in soft tissue swelling, bone callus proliferation, and consolidation of fractures. Finally, the *reconstructive* stage is denoted by bony ankylosis and hypertrophic proliferation with some restoration of stability when this stage is reached. In certain cases, however, severe osseous disintegration occurs due to prolonged activity. In these situations the condition may be referred to as chronically active and little healing, if any, takes place. While the system is radiologically very descriptive and useful, its practical clinical applicability is less so. In clinical practice, the initial developmental stage is considered *active* or *acute*, while the coalescent and reconstructive stages are considered to be the *inactive* or *quiescent* stages. Other classification systems have been described based upon anatomic sites of involvement but do not describe the activity of the disease [46, 57, 70–72]. Rogers and Bevilacqua described a prognostic staging system based on anatomic location and complicating factors of the Charcot joint (Fig. 22.17) [72, 73], which was later validated by Viswanathan et al. in a group of 53 patients [74]. The Sanders and Frykberg classification is descriptive, based on the site of involvement and was described in detail above [2].

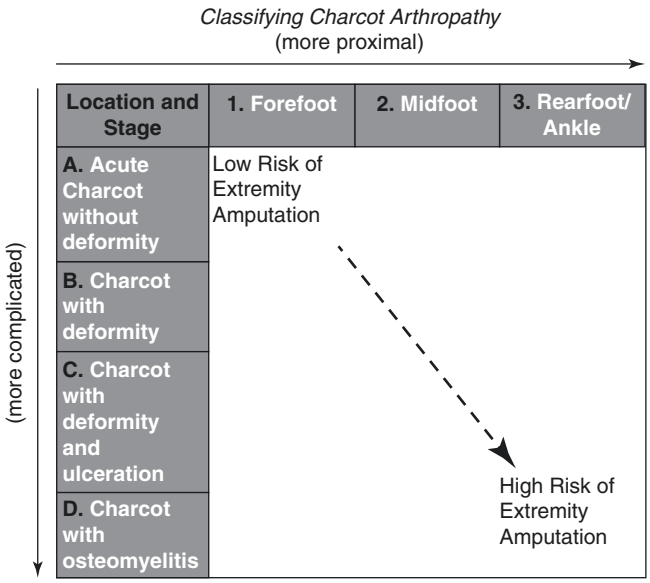

Fig. 22.17 Rogers and Bevilacqua dual axis classification of Charcot foot based upon location and complications. (From Lee C. Rogers, with permission)

The American Diabetes Association and American Podiatric Medical Association created the Joint Task Force on the Charcot Foot comprised of a multinational group of Charcot foot experts in 2010. Given the confusion about classifications, their limited prognostic value, and inability to direct treatment, the Joint Task Force recommended simplifying the clinical classification of the Charcot foot to *active* or *inactive* based on the presence of inflammation [44].

Medical Management

Offloading

Immobilization and reduction of stress are considered the most important treatment for active Charcot arthropathy [17, 44]. Effective offloading or complete non-weight-bearing on the affected limb removes the continual trauma and should promote conversion of the active Charcot joint to the inactive quiescent phase [17, 40, 54]. Non-weight-bearing is an accepted form of offloading for most foot and ankle injuries; however, three point crutch gait may increase pressure to the contralateral limb, thereby predisposing it to repetitive stress and ulceration or an active Charcot episode [56]. Additionally, those with diabetes and neuropathy tend to be older and overweight and do not have the cardiovascular reserve for the additional energy required to use crutches effectively. A patient with neuropathy severe enough to lead to a Charcot foot is likely to have proprioceptive impairment and had an increased risk of falling with offloading treatment, especially crutches. Since total non-weight-bearing is frequently unattainable for many patients in this category, total contact casts (TCC) may serve as a useful alternative and is the most effective form of offloading while bearing weight [75]. TCC can be applied safely in those with Charcot foot, but should be changed frequently at first since edema tends to reduce greatly with immobilization and offloading which will lead to a poorly fitting cast [44]. Figure 22.18 shows the major mechanisms of action of a total contact cast in relieving plantar pressure and immobilizing the foot and ankle. In those patients where a TCC cannot be used, a soft compressive dressing or Unna's Boot in concert with a removable cast walker or pneumatic walking brace can also be used secondarily in this regard [30]. However, a large study in the United Kingdom found that in patients using removable devices took significantly longer to heal versus the TCC group [18]. In the presence of ulcers or infections, frequent debridements and careful observation are required.

Offloading and immobilization should be anticipated for approximately for 6 months or more, depending on the severity of joint destruction. Conversion to the inactive/reparative phase is deduced by a reduction in pedal temperature to

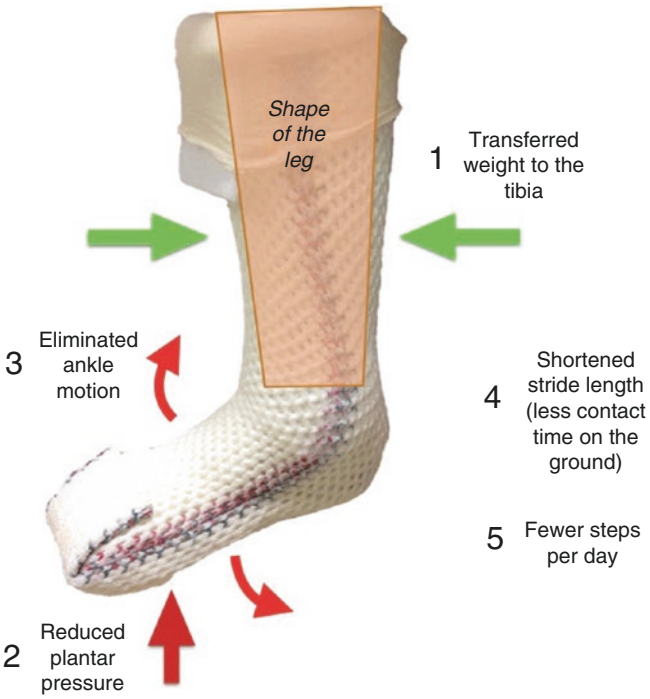

Fig. 22.18 An image of a total contact cast (TCC) depicting the major mechanisms of action in offloading and immobilization. (From Lee C. Rogers, with permission)

Table 22.4 Offloading/immobilizing devices used in the management of Charcot feet

• Total contact cast (TCC)
• Wheelchair
• Crutches
• Rolling knee walker
• Removable cast walker (RCW)
• Patellar tendon-bearing orthosis (PTBO)
• Charcot restraint orthotic walker (CROW)

within 4 °F (2.5 °C) of that of the unaffected side and a sustained reduction in edema [15]. This should be corroborated with serial radiographs indicating consolidation of osseous debris, union of fractures, and a reduction in soft tissue edema. McGill et al. have found a reduction in skin temperature and bone scan activity that mirrors activity of Charcot neuroarthropathy, both of which improve as the condition achieves the inactive stage or quiescence [43].

When the patient enters the inactive stage, management is directed at a gradual resumption of weight-bearing with prolonged or permanent bracing [15, 17, 44]. Care must be taken to gradually wean the patient from non-weight-bearing (or TCC) to partial to full weight-bearing with the use of assistive devices (i.e., crutches, cane, or walker). Progression to *protected* weight-bearing is permitted, usually with the aid of some type of ambulatory, immobilizing device (Table 22.4) [76]. Charcot restraint orthotic walkers ("CROW") or other similar total contact prosthetic walkers have gained acceptance as useful protective modalities for the initial period of weight-bearing after TCC [77]. These custom-made braces usually incorporate some degree of patellar tendon bearing as well as a custom footbed with a rocker sole. A more readily available option is a pneumatic walking brace or similar removable cast walker that might incorporate a cushioned footbed or insole. These can be made less-removable or non-removable by simply applying adhesive tape or cast bandaging around the body of the brace to help encourage compliance (Fig. 22.19) [78].

The mean time of rest and immobilization (casting followed by removable cast walker) prior to return to permanent protective footwear is approximately 4–12 months [15, 18, 40]. Feet must be closely monitored during the time of transition to permanent footwear to insure that the acute inflammatory process does not recur. Forefoot and midfoot deformities often do well with custom full-length inserts and comfort or extra-depth shoes once bracing is no longer

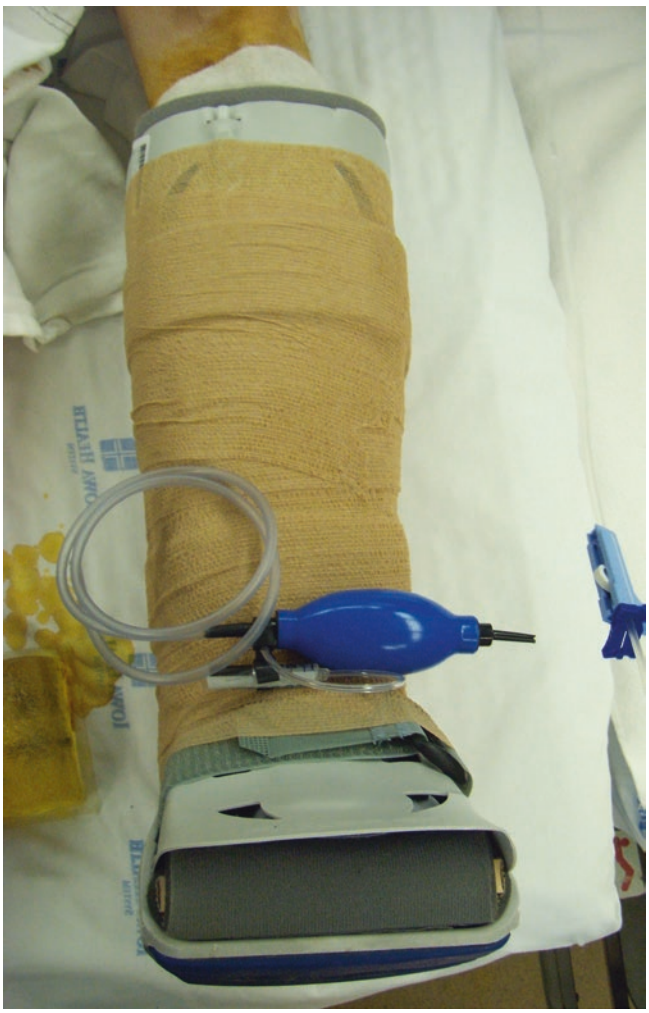

Fig. 22.19 A removable cast walker (RCW) rendered "less removable" with an external layer of cohesive bandage. (From Lee C. Rogers, with permission)

required [17]. Continuing effective offloading with non-custom bracing or TCC often serve as interim footwear prior to obtaining permanent custom-made footwear. Severe midfoot deformities will often require the fabrication of custom shoes to accommodate the misshapen foot. Rearfoot neuroarthropathy with minimal deformity may require only a deep, well-cushioned shoe with a full-length orthotic device. For mildly unstable ankles without severe deformity or joint dissolution, high-top custom shoes can sometimes provide adequate stability against transverse plane rotational forces. The moderately unstable ankle will benefit from an ankle foot orthosis (AFO) and a high-top therapeutic shoe. The severely unstable or maligned rearfoot will require a patellar tendon bearing (PTB) brace incorporated into a custom shoe [79, 80]. The PTB brace has reportedly decreased the rearfoot mean peak forces by at least 32% [80].

Anti-resorptive Therapy

In the setting of altered bone mineral density (BMD) in patients with diabetes and neuropathy, there has been recent interest in the adjunctive use of bisphosphonate therapy in acute Charcot arthropathy [28, 36, 81, 82]. However, further study has cast a negative shadow on their routine use for Charcot foot [18] and it is not recommended by the Joint Task Force [44]. These pyrophosphate analogs are potent inhibitors of osteoclastic bone resorption and are widely used in the treatment of osteoporosis, Paget's disease, and reflex sympathetic dystrophy syndrome. Although one uncontrolled study of six patients found significant reductions in foot temperature and alkaline phosphatase levels as compared to baseline, its small size and lack of a control group preclude making any meaningful conclusions from the treatment [82]. A subsequent multicenter randomized trial in the UK from this same group was performed using a single intravenous infusion of pamidronate compared to saline infusion [36, 82]. The treatment group had significant falls in temperature and markers of bone turnover (deoxypyridinoline crosslinks and bone specific alkaline phosphatase) in subsequent weeks as contrasted to the control subjects. However, no differences in clinical or radiographic outcomes were reported. Trials of oral bisphosphonates with alendronate have been done but the effects of the treatment take up to 6 months which is not likely sufficient in this limb-threatening disorder requiring more urgent action [83]. Until definitive controlled outcome studies are performed which concurrently measure serum markers of osteoclastic activity and attempt to assess improvements in clinical and radiological healing, and based on further clinical outcomes study, the routine use of bisphosphonate therapy should be avoided.

Another pharmacologic agent interrupting the bone resorptive pathway which has been investigated in Charcot foot is intranasal calcitonin. It is often used for osteoporosis has been shown to reduce markers of bone turnover and foot temperature differences in Charcot foot [84]. Some have theorized that it has a direct effect on RANK-L and may interrupt the deposition of calcium from the bone to the intima media of the blood vessels [37].

Bone Stimulators

Another modality which has been applied to the management of acute neuroarthropathy is the use of bone stimulation [85–87]. In one study of 31 subjects randomized to either casting alone or cast with Combined Magnetic Field (CMF) electrical bone stimulation, there was a significant reduction in time to consolidation of the Charcot joints in the study group (11 vs. 24 weeks) [86]. Low intensity pulsed ultrasound (LIPUS) has also been suggested as a useful adjunct in promoting healing of Charcot fractures, although this report only presented two cases of patients successfully treated after undergoing revisional surgery for recalcitrant deformities [88]. While both types of modalities have been proven successful in healing chronic nonunions or even fresh fractures (in the case of LIPUS), their efficacy in promoting prompt healing of acute Charcot fractures or union of surgical arthrodeses has yet to be proven by large, well-controlled randomized clinical trials. Direct current implantable bone stimulators have shown benefit in Charcot foot reconstruction with arthrodesis [89].

Surgical Treatment

The Charcot foot should not be considered as primarily a surgical disorder, with a few exceptions. There is an abundance of support in the literature confirming the need for initial attempts at medical treatment, including offloading, to arrest the destructive process by converting the active Charcot joint to its inactive state [17, 40, 44, 79]. The Joint Task Force produced a treatment algorithm when considering nonsurgical versus surgical treatment (Fig. 22.20). As indicated by Johnson in 1967, the three keys to treatment of this disorder should be prevention first, followed by early recognition, and once diagnosed, protection from further injury until all signs of "reaction" have subsided [34]. Surgery should be contemplated when attempts at medical treatment as previously outlined have failed to provide a stable, plantigrade foot or in cases of gross dislocation. Additionally, when uncontrollable shearing forces result in recurrent plantar ulcerations or in those unusual cases that demonstrate continued destruction despite non-weight-bearing, procedures such as simple bone resections, osteotomy, midfoot or major tarsal reconstruction, and ankle

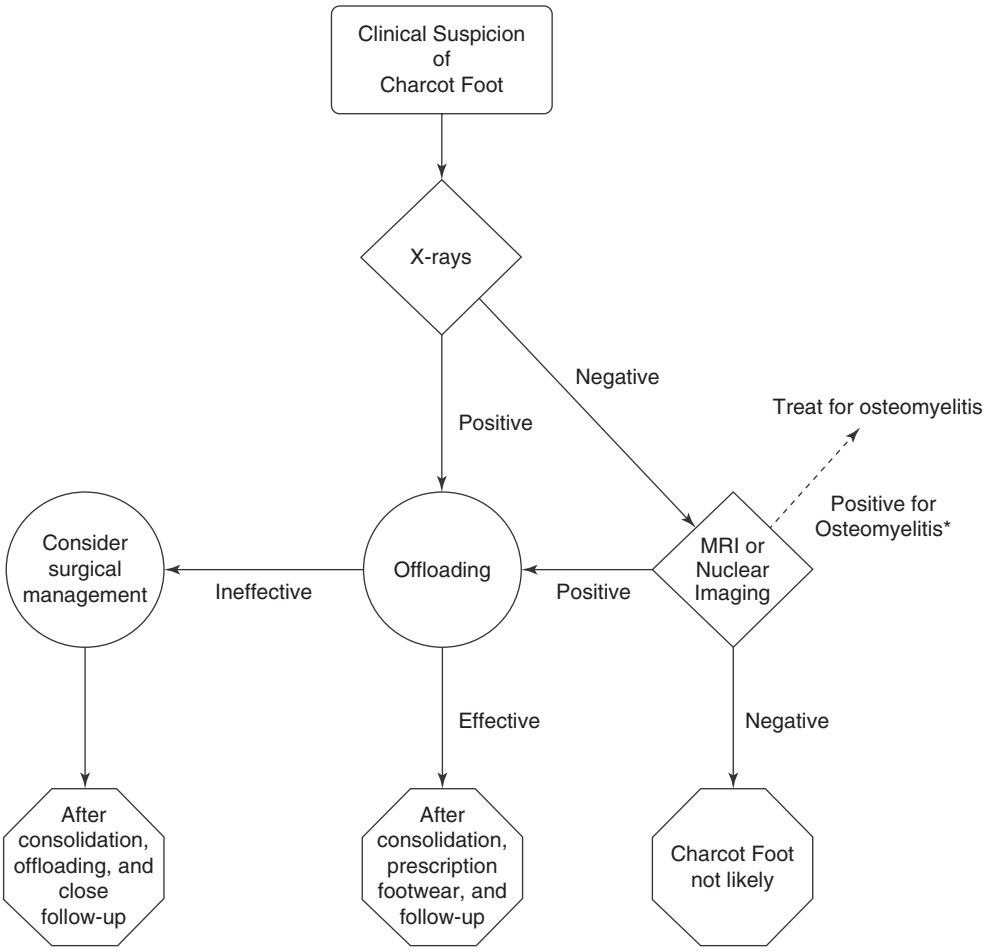

Fig. 22.20 The American Diabetes Association/ American Podiatric Medical Association Charcot Foot Joint Task Force treatment algorithm. (From Rogers LC, et al. The Charcot foot in diabetes. J Am Podiatr Med Assoc 2011;101:437–46, with permission)

arthrodesis might become necessary [44]. However, a recent review of one center's experience with midfoot neuroarthropathy in 198 patients (201 feet) indicated that more than half of these patients could be successfully managed without the need for surgery [76].

Although becoming more common in clinical practice, surgery on the Charcot foot is not a new concept, but there is still little good quality evidence to show its value [90]. Steindler, in 1931, first reviewed his series of operative results in tabetic patients including one subtalar arthrodesis [91]. He, like Samilson [92], Harris and Brand [57], and Johnson [34] many years later, recommended early recognition of the arthropathy, immediate protection from external deforming forces, and early operative stabilization when significant malalignment and instability precluded further conservative treatment. Samilson in 1959 [92] and Heiple in 1966 [93] were early to recognize the necessity for compressive internal fixation and prolonged immobilization in effectuating a solid bony fusion.

Harris and Brand in 1966 provided insight into this disorder associated with leprosy and described their five patterns of "disintegration of the tarsus" [57]. Full immobilization was always deemed imperative as an initial treatment; however, when progression continued or an unsatisfactory result was obtained, early surgical fusion was advocated. One year later Johnson published his large series which established the need for early recognition and protection to allow the acute inflammatory response to subside prior to surgical intervention [34]. As he stated, "Appropriate surgery on neuropathic joints, performed according to these principles, should be undertaken with great respect for the magnitude of the problem but not with dread." Johnson clearly favored osteotomy or arthrodesis in selected patients with quiescent Charcot joints and deformity in order to restore more normal alignment. Since the trauma of surgery could result in further absorption of bone during the acute stage, great emphasis was placed on resting the part until there was clinical and radiographic evidence of repair. Only then could surgery be attempted with a favorable chance for success [34].

Indications and Criteria

Instability, gross deformity, and progressive destruction despite immobilization are the primary indications for surgical intervention in neuroarthropathy [17, 34, 44, 94].

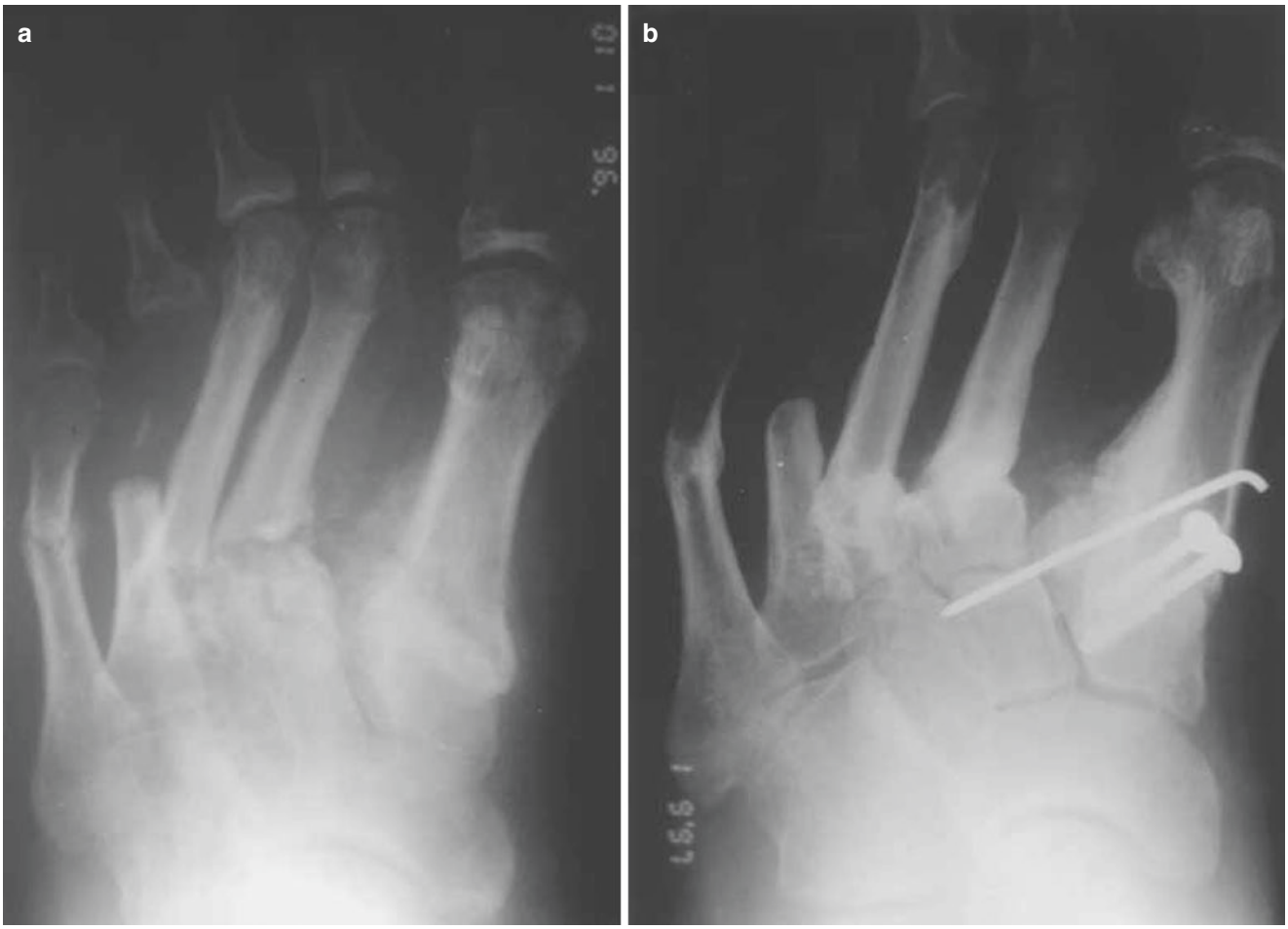

Fig. 22.21 (**a**) Preoperative X-ray of patient with dorsally dislocated first metatarsal-cuneiform joint and several metatarsal fractures. (**b**) Stability, resolution of symptoms, and complete healing was achieved with a limited arthrodesis of the first ray

Additionally, recurrent ulceration overlying resultant bony prominences of the collapsed rear, mid, and forefoot may require partial ostectomy to effect final healing when performed in conjunction with appropriate footwear therapy [95, 96]. Pain or varying degrees of discomfort will frequently accompany the deformity and may be refractory to medical treatment in some patients. Attributable to chronic instability, this can be effectively eliminated by limited arthrodeses at the primary focus of the neuroarthropathy (Fig. 22.21).

Lesko and Maurer[56] and Newman [55, 97] in their considerations of spontaneous peritalar dislocations advocate primary arthrodesis in those acute cases where there is a reducible luxation in the absence of significant osseous destruction. Since these luxations may be the initial event in the sequence leading to typical osteoarthropathy, early intervention following a period of non-weight-bearing has been recommended to counteract forces which would most likely lead to further progression of the deformity.

Age and overall medical status should also weigh heavily in the decision regarding suitability for surgery. Recognizing that arthrodesis and major reconstructions will require cast immobilization and non-weight-bearing for 6 months or more, selection of the appropriate patient is critical to a successful outcome [98–100]. Since the majority of patients with osteoarthropathy are in their sixth to seventh decades and may likely have coexistent cardiovascular or renal disease, careful consideration must be given to the risk versus benefit of lengthy operative procedures and the attendant prolonged recuperation [42]. As mentioned, a simple bone resection or limited arthrodesis might suffice in an older patient with a rocker-bottom deformity prone to ulceration as opposed to a complete reconstruction of the midfoot [96, 101]. The former procedures can be done under local anesthesia relatively quickly, require a shorter convalescence, are prone to fewer complications, and can provide a stable, ulcer-free foot when maintained in protective footwear. Nevertheless, major foot reconstructions and arthrodeses are certainly indicated in those healthier patients with severe deformity, instability, or recurrent ulcerations who have not satisfactorily responded to medical treatment [44, 100] (Fig. 22.22). In all cases, however, the patient must be well

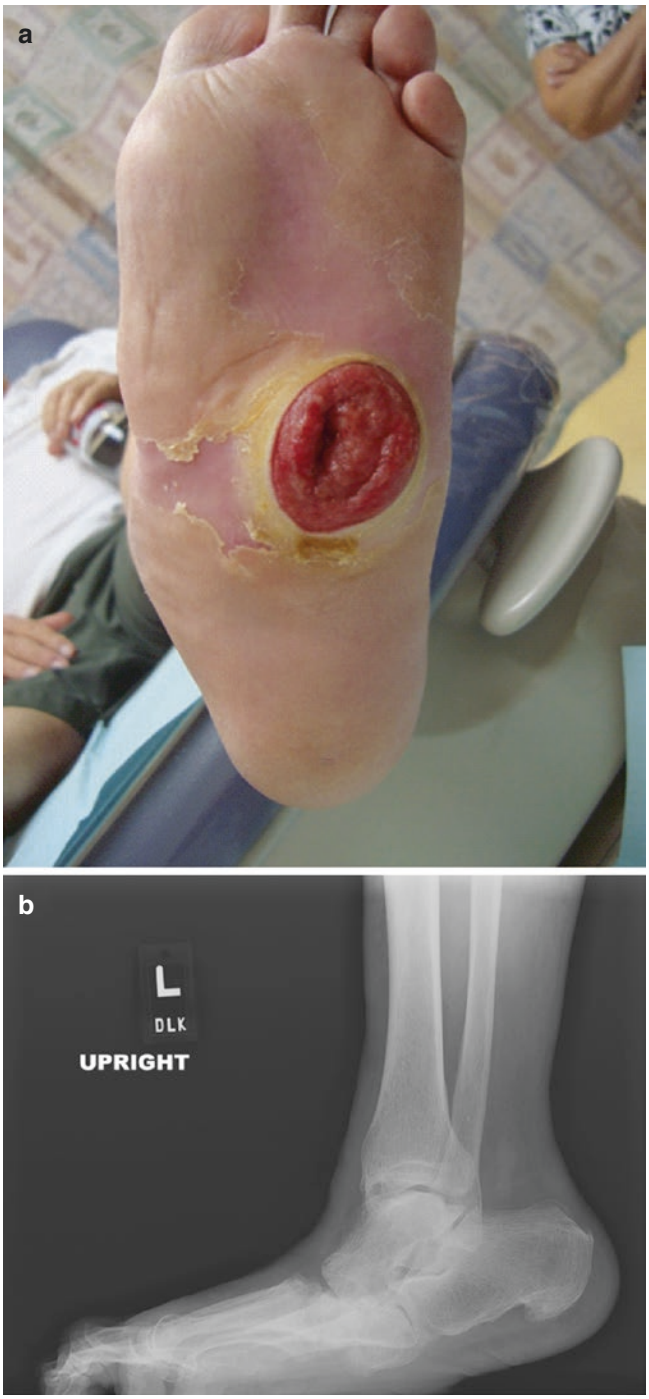

Fig. 22.22 (**a**) This patient has a chronic midfoot ulcer associated with a rocker-bottom deformity. (**b**) Radiograph of same patient showing severe rearfoot equinus and midfoot deformity

educated as to the necessity for strict compliance with postoperative immobilization and non-weight-bearing or partial weight-bearing for as long as 6–12 months.

An acute deformity, either a spontaneous dislocation or the more advanced fracture-dislocation paradigmatic of neuroarthropathy, is generally rested and immobilized prior to any attempted surgery. Surgery during the active stage has the potential to compound and exacerbate the bone atrophy indicative of this inflammatory stage of destruction. Hence, it may be counterproductive as well as detrimental to operate on these feet until they have been converted to the quiescent, reparative stage. One small series, however, indicates successful arthrodesis rates with preserved foot function in patients with acute arthropathy of the midfoot [102]. Others have also advocated early operative repair with arthrodesis during stage 0 or stage 1, especially when nonoperative treatment has failed to prevent further deformity or arrest the destructive process [103–105]. Notwithstanding, this aggressive surgical approach needs confirmation through larger comparative trials prior to its adoption in the routine management of the acute Charcot foot.

Surgical Procedures

Surgery performed primarily on chronic Charcot feet has met with increased success in recent years as experience develops and improvements in fixation are made. With an average union rate of 70% and improved alignment with stability, surgery on the Charcot foot has the potential not only to save limbs but also to improve quality of life [45]. Surgical correction of the Charcot foot can be segregated based on complexity, with the simpler surgeries having fewer complications (Fig. 22.23).

Ostectomy of plantar prominences in the face of recalcitrant or recurrent neuropathic ulceration is perhaps the most frequent procedure performed on Charcot feet [96]. Such operations are fairly easy to perform and do not generally require lengthy periods of immobilization beyond attaining wound closure. Surgical approaches are varied, with direct excision of ulcers by ellipse or rotational local flaps predominating. Alternative incisions are performed adjacent to ulcers or prominences, either through a medial or lateral approach. One report suggests that excision of medial plantar prominences fare better and with fewer complications

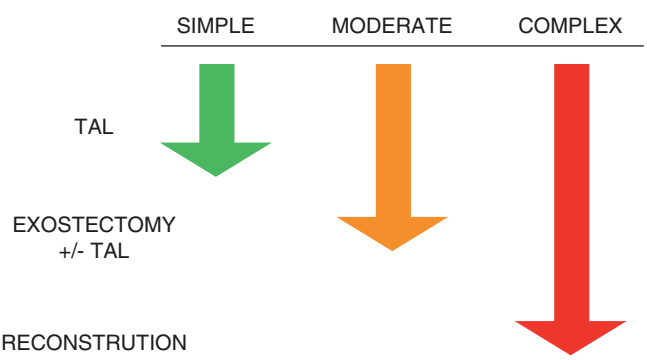

Fig. 22.23 A chart of the scaling complexity of Charcot foot surgeries. *TAL* tendo-Achilles lengthening. (From Lee C. Rogers, with permission)

than those under the lateral midfoot [96]. However, an earlier study reviewing experience with only lateral column ulcers reported an 89% overall healing rate [101]. A flexible approach to both incision and soft tissue coverage, including tissue transfer, is therefore required for optimal outcomes in cases of midfoot plantar ulceration.

Arthrodesis of unstable Charcot joints of the midfoot and rearfoot frequently becomes necessary to provide a useful, plantigrade foot in those situations where bracing or footwear therapy have been unsuccessful [17, 44, 100, 106]. Major foot reconstruction is also an attractive alternative to amputation in patients with chronic or recurrent ulceration. Thompson et al. recommend reconstructive surgery for Charcot deformities unable to function with load sharing orthoses [107, 108]. Commonly, a tendo-Achilles lengthening precedes the fusion to ultimately diminish the plantarflexory forces contributing to pedal destruction [17, 109]. The traditional method for arthrodesis has been open reduction with solid internal fixation for uninfected Charcot joints, while external fixation is utilized when there is suspected infection of the joint fusion site [100]. In recent years, however, there has been greater interest in using external fixation and circular (Ilizarov) frames for stabilization in the Charcot foot of acute and chronic durations, and for maintenance of correction for major reconstructions (Fig. 22.24) [110–112]. Proposed benefits of circular frames include their ability to maintain fixation even in osteopenic bone, early weight-bearing ability, avoidance of fixation devices at sites of ulceration and potential bone infection, the ability to correct severe deformities, and the capability for gradual adjustments in position and compression throughout the reparative process [110]. For ankle deformities requiring arthrodesis, some prefer to use retrograde intramedullary nails alone or in concert with external fixators to provide stability and enhanced rates of fusion [113–115].

Operative fusion techniques vary by site, but generally require meticulous excision of the synovium, resection of sclerotic bone down to a healthy bleeding bed, open manipulation, and precise osteotomies prior to rigid fixation. Tissue handling must be gentle to avoid undue trauma and dissection must be mindful of underlying neurovascular structures. After reduction of deformity temporary fixation is achieved with large Steinman pins, K-wires, or guide pins when cannulated screw systems are to be used [109]. After copious lavage, a surgical drain is placed before primary wound closure. External circular frames are generally constructed preoperatively and then applied with appropriate technique after wound closure.

Newer research studying dynamic peak plantar pressures pre- and postoperatively shows promise in proving that surgical reconstruction of the Charcot foot is beneficial (Fig. 22.25) [116].

Postoperative to internal fixation procedures, the patient immediately undergoes immobilization of the foot with a posterior splint or bivalve cast. The patient must adhere to strict bed rest and prevent lower extremity dependency for several days until the soft tissue swelling subsides and serial below knee casting begins. The patient will remain non-weight-bearing for a minimum of 2–3 months prior to considering partial weight-bearing. In general, protected weight-bearing should be the rule for 6–12 months in order to avoid nonunion or late deformity in these difficult patients. After external fixation weight-bearing status is variable. Some surgeons allow limited or full weight-bearing while others choose to keep patients non-weight-bearing while the frame is in place. The contralateral extremity should be protected from the components of the external fixator which could cause injury. This can be accomplished by covering the external fixator or the contralateral extremity [117]. Advancement to weight-bearing cast, total contact cast, or walking brace will follow after evidence of consolidation. One reasonable approach is to remove the fixator after 2 months with subsequent application of an ambulatory total contact cast for several more months until there is evidence of radiographic consolidation [106]. Once healed, therapeutic footwear with or without bracing is necessary to prevent recurrent foot lesions.

Complications

Traditionally, surgery on neuropathic joints had been met with a good deal of failure including high rates of nonunion, pseudoarthrosis, and infection. Most such occurrences can now be attributed to a failure of appreciation of the natural history of osteoarthropathy and lack of attention to the necessary criteria and the basic tenets of surgery on Charcot joints as previously discussed. Even with this knowledge, however, complications can ensue in these high-risk feet during the immediate postoperative period and beyond.

Infection can be a major sequel of surgery and of course can threaten the success of an attempted arthrodesis site as well as the limb itself. Most longitudinal studies and reports of surgery on the Charcot foot indicate a certain percentage of patients in whom osteomyelitis or severe infection developed that necessitated major amputation [15, 42]. Therefore, caution must constantly be exercised in these patients to ensure that infection or osteomyelitis is controlled and eradicated prior to reconstructive surgery. Perioperative antibiotic therapy is certainly indicated in these compromised patients and once present, infection must be aggressively treated. With the use of external fixators comes the risk of pin tract infections or wire breakages requiring further surgery [118, 119]. But if complications are managed on a proper and

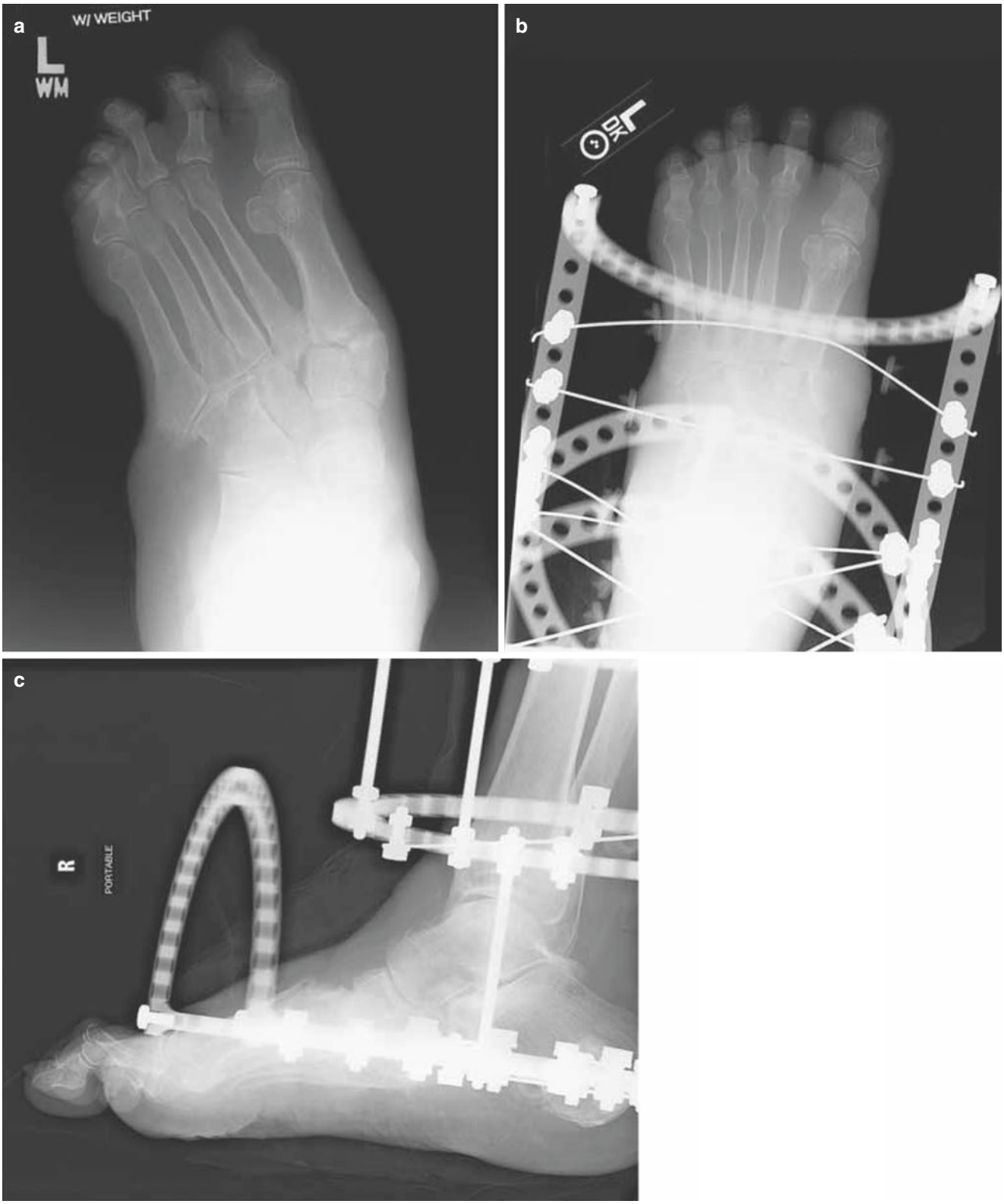

Fig. 22.24 Midfoot Charcot deformity corrected with circular external fixation. (**a**) Preoperative AP view showing midfoot deformity. (**b**) Postoperative AP view showing correction and frame in place. (**c**) Lateral postoperative X-ray with circular frame in place

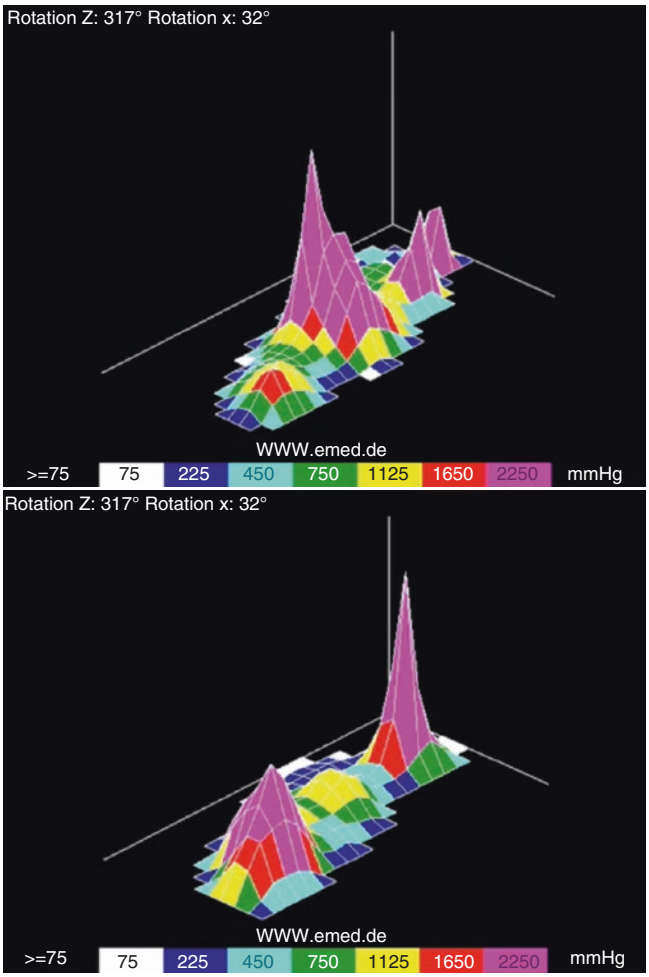

Fig. 22.25 Illustration of dynamic peak plantar measurements in a patient before (top) and 6 months after (bottom) Charcot foot reconstruction. Note the resolution of high plantar midfoot pressures postoperatively and the return of a more normal pattern, which includes higher pressure under the heel and forefoot. (From Lee C. Rogers, with permission)

timely basis, their presence does not change the outcome of the surgery.

Pseudoarthrosis and nonunion are very troublesome complications in non-neuropathic patients undergoing arthrodesis or osteotomy. However, this is not always the case in neuropathic patients undergoing the same type of reconstructive procedures. As long as stability and satisfactory alignment are achieved, a failure of complete arthrodesis or union is not necessarily considered to be a failure of surgery [109, 110]. Just as they will not sense the discomfort of post-traumatic arthritis in unreduced fracture-dislocations, these patients will have no symptoms from a stable, well-aligned nonunion. Nonetheless, the surgical principles for achieving solid union as previously discussed must always be followed when operating on these patients.

Since the trauma of surgery in itself can potentially incite an acute active reaction in a chronic inactive neuropathic joint, one must always treat the newly operated foot as an active Charcot joint. Furthermore, Clohisy makes a strong argument for prophylactic immobilization of the contralateral extremity to prevent the development of an acute deformity on the supporting foot [120]. Ablative or corrective procedures of the forefoot can also have detrimental effects on adjacent structures as well as on the midfoot and rearfoot. Biomechanical alterations will result in increased areas of vertical and shear stress in new sites which will then be predisposed to ulceration and neuroarthropathy. Therefore, surgery of any kind on the neuropathic foot must be performed with discretion and with attention to proper postoperative care to obviate the occurrence of these potentially destructive sequelae.

Amputation should usually be regarded as a procedure of last resort in neuropathic patients and not as a normal consequence of osteoarthropathy. While this outcome can sometimes represent a failure in early recognition and management, amputation usually results from overwhelming postoperative infection or late stage ulcerations. Unfortunately, amputation will always be a necessary consideration in this complicated group of patients [121]. In certain situations, amputation might be the *best* alternative to a difficult reconstruction in an unstable patient or in those patients who do not wish to engage in the lengthy recuperative period that follows a major arthrodesis. However, this is generally reserved for those extremities beyond salvage after all other attempts at medical and reconstructive care have failed.

Conclusion

The Charcot foot is a very serious limb-threatening complication of diabetes that can be attributed to preexisting peripheral neuropathy compounded by some degree of trauma. Oftentimes the diagnosis is missed which can lead to further destruction [122, 123]. The attendant hypervascular response coupled with osteopenia, fractures, and dislocations can rapidly evolve into severe foot deformities as a consequence of continued weight-bearing. It is therefore incumbent upon both the patient to seek early consultation and the practitioner to diagnose the process early in order to arrest the progression of the destructive phase and institute appropriate treatment. While non-weight-bearing and immobilization remain the most effective treatment in the active stage, over the last decade there has been greater interest in surgical solutions for the severe deformities, recurrent ulcers, or instability. As our knowledge and experience have grown, long-term outcomes have improved. As of yet, however, many questions remain unanswered pertaining to the precise mechanisms involved in the etiology of neuroarthropathy as well as those concerning optimal early and late stage treatments. With a heightened suspicion for the disorder, further prospective research, and an evidence-based approach to treatment, the future holds even greater promise for these patients.

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Amputations and Rehabilitation

23

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Abstract

An amputation of the lower extremity is erroneously considered a failure of conservative care or an unpreventable outcome of diabetes. In the diabetic population, a lower extremity amputation is often the result of ischemia or uncontrolled infection. This chapter will discuss multiple factors that should be evaluated to optimize the outcome of any amputation. The technique and important intraoperative factors when performing an amputation are discussed. Following an amputation, a rehabilitation process is begun to return the patient back into the community. Discussed are the factors that influence a patient's rehabilitation potential as a community ambulator.

Indications and Basic Principles of Amputation

Amputation of the foot may be indicated when neuropathy, vascular disease, and ulcerative deformity have led to soft tissue necrosis, osteomyelitis, uncontrollable infection, or intractable pain.

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Amputations of the lower extremity are often considered either a failure of conservative management or an unpreventable outcome of diabetes. The patient views amputation as the end of productivity and the start of significant disability. Amputation should be viewed as a procedure, leading to rehabilitation and return to productivity for the patient disabled by an ulcerated, infected, or intractably painful extremity. The patient needs assurance and the goal is to return the patient to productive community activity. This may involve consultation among the specialties of medicine, podiatry, orthopedics, vascular surgery, interventional radiology/cardiology, physiatrists, and prosthetists. As the patient is rehabilitated and returns to the activities of daily living, the residual limb and the contra lateral limb must be protected. Revision amputation and amputation of the contra lateral limb remain a significant problem, occurring in as many as 20% of amputee cases [1].

Significant reductions in amputation rates occurred from the middle 1990s until 2010. This reduction was related to improvements in vascular intervention methods for ischemic patients and access to care for these procedures (Fig. 23.1). Amputation rates for nonischemic pathologies have remained quite level over this same timeframe [2].

The goal of any limb salvage effort is to convert the diabetic foot to a Wagner grade 0 extremity. Those patients with Wagner grade 5 foot will require an appropriate higher level of amputation. If salvage is not feasible, then all efforts are made to return the patient to some functional level of activity after amputation. The more proximal the amputation, the higher the energy cost of walking. The most significant problem our patients face is multisystem disease and limited cardiopulmonary function. These factors may negatively impact the patient's postoperative independence.

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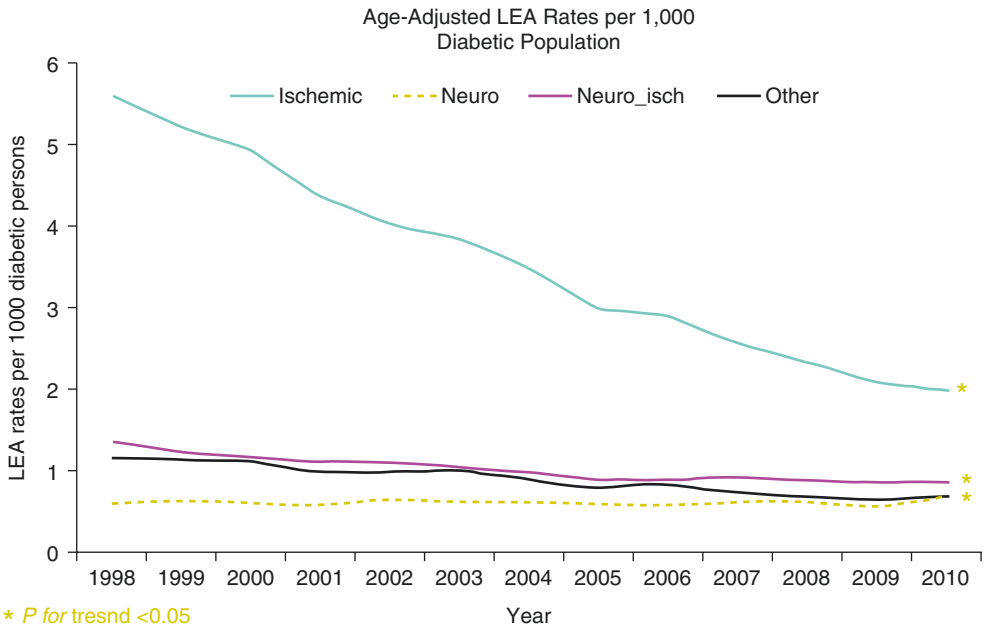

Fig. 23.1 This table reflects the vast improvements in vascular intervention on amputation rates of the lower extremity. Other than vascular intervention, no significant improvements in amputation rates have been made for other causes of amputation

Limb Salvage Versus Limb Amputation

Patients may require several surgical treatments before definitive amputation. Incision and drainage or open amputation is frequently required to stabilize acute infection. The parameters of healing, to be mentioned later, may not apply at that time. The goal of the first stage of a multi-staged procedure is simply to eradicate infection and stabilize the patient. If medical review of the patient suggests an inability to tolerate multiple operations, a more proximal initial level of amputation may be indicated foregoing attempts at distal salvage. However, if salvage is possible, and the patient is medically stable, then a systematic approach to limb salvage should be pursued.

Enlightened orthopedic care of the new millennium has changed focus from results to outcomes. Burgess taught us that amputation surgery is the first step in the rehabilitation of a patient with a nonfunctional or reconstructable limb [3]. The desired outcome is the reentry of the amputee into their normal activities, while also setting achievable functional goals.

Lower extremity amputation is performed for ischemia, infection, trauma, neoplastic disease, or congenital deformity. The following concerns should be addressed before undertaking either an attempt at limb salvage, or performing an amputation:

1. Will limb salvage outperform amputation and prosthetic limb fitting? If all transpires as one could reasonably predict, will the functional independence of the patient following limb salvage/reconstruction be greater, or less than, amputation and prosthetic limb fitting? This will vary greatly with age, vocational ability, medical health, lifestyle, education, and social status.
2. What is a realistic expectation of functional capacities at the completion of treatment? A realistic appreciation of functional end results should be made with respect to both limb salvage and amputation. Consultation with physical medicine and rehabilitation, social work, and physical therapy can assist in determining reasonable outcome expectations.
3. What is the time and effort commitment required for both the treatment team and the patient? Both the physician and patient must have a reasonable understanding of the duration of the rehabilitation process, the inherent risks involved with revascularization, and the effort required for both.
4. What is the expected financial cost to the patient and resource consumption of the healthcare system? Direct expenses of diabetic foot ulceration and amputations were estimated to cost US healthcare payers $10.9 billion in 2001 and increased to $116 billion in 2007. Indirect expense (disability, work loss, and premature mortality) was estimated at $58 billion [4, 5].

Physical and Metabolic Considerations

Metabolic Cost of Amputation

The metabolic cost of walking is increased with proximal level amputations, being inversely proportional to the length of the residual limb and the number of joints preserved.

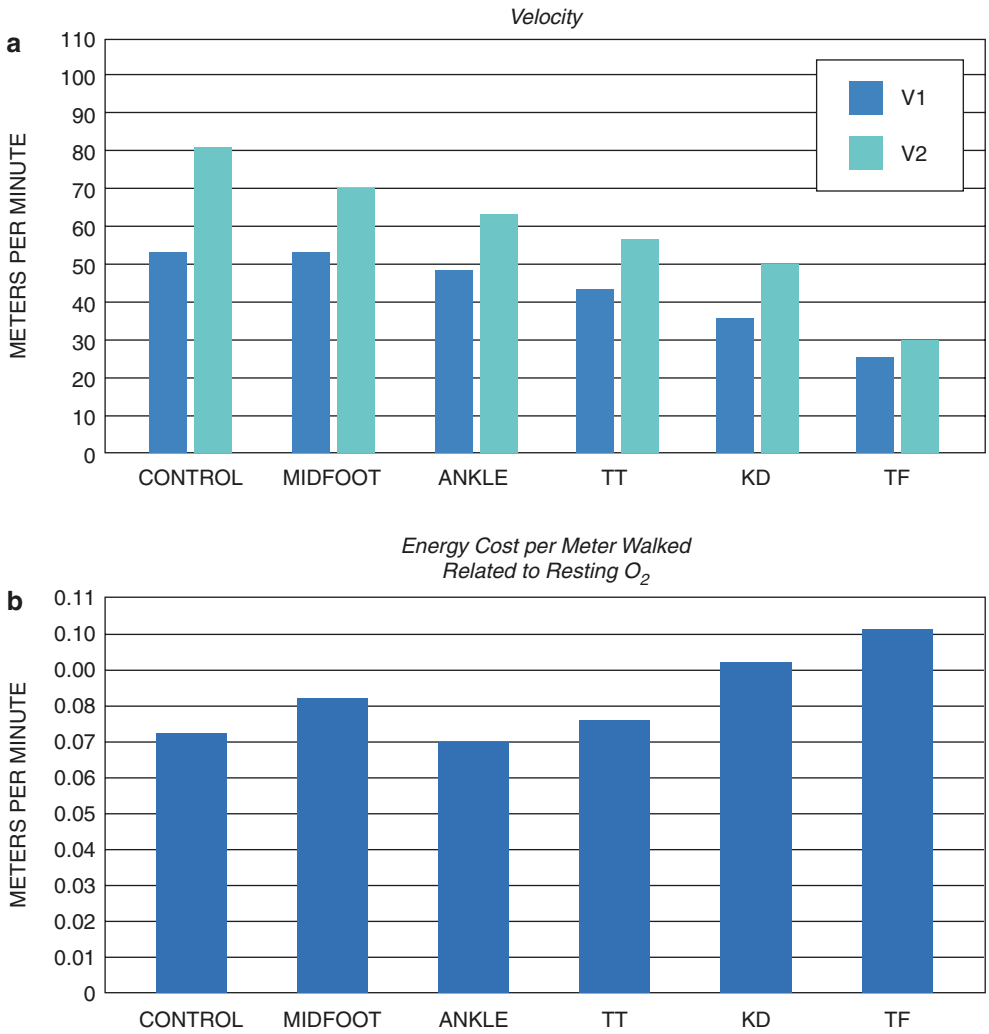

Fig. 23.2 Table of velocity and energy cost. (**a**) Walking velocity compared to surgical amputation level. V1 is self-selected walking speed. V2 is maximum walking speed. (**b**) Oxygen consumption per meter walked compared to surgical amputation level. Note that the metabolic cost of walking is increased with more proximal level amputation

With more proximal amputation, patients have a decreased self-selected, and maximum, walking speed. Oxygen consumption is also increased. From an outcomes perspective, functional independence (functional independence measure score) is directly correlated with amputation levels. Distal level amputees achieve proportionally higher functional independence measure scores (Fig. 23.2) [6–8].

Cognitive Considerations

It is suggested that many individuals with long-standing diabetes have cognitive and perceptual deficits (Fig. 23.2) [9–13]. There are certain specific cognitive capacities that are necessary for individuals to become successful prosthetic users: memory, attention, concentration, and organization. In order for patients with these deficiencies to become successful prosthetic users, they require either successful education and training, or the physical presence of a caregiver that can provide substitute provision of these skills.

Load Transfer and Weight Bearing

Feet act as uniquely adapted end organs of weight bearing. Following amputation, the residual limb must assume the tasks of load transfer, adapting to uneven terrain, and propulsion, utilizing tissues that are not biologically engineered for that purpose. The weight-bearing surfaces of long bones are wider than the corresponding diaphysis. This increased surface area dissipates the force applied during weight bearing over a larger surface area, and the more accommodative articular surface and metaphyseal bone allow better cushioning and shock absorption during weight bearing.

Direct load transfer, i.e., end bearing, which is achieved in disarticulation amputations at the knee and ankle joint levels, takes advantage of the normal weight-bearing characteristics of the terminal bone of the residual limb. The overlying soft tissue envelope acts to cushion the bone, much as the heel pad and plantar tissues function in the foot.

Indirect load transfer, or total contact weight bearing, is necessary in diaphyseal transtibial and transfemoral

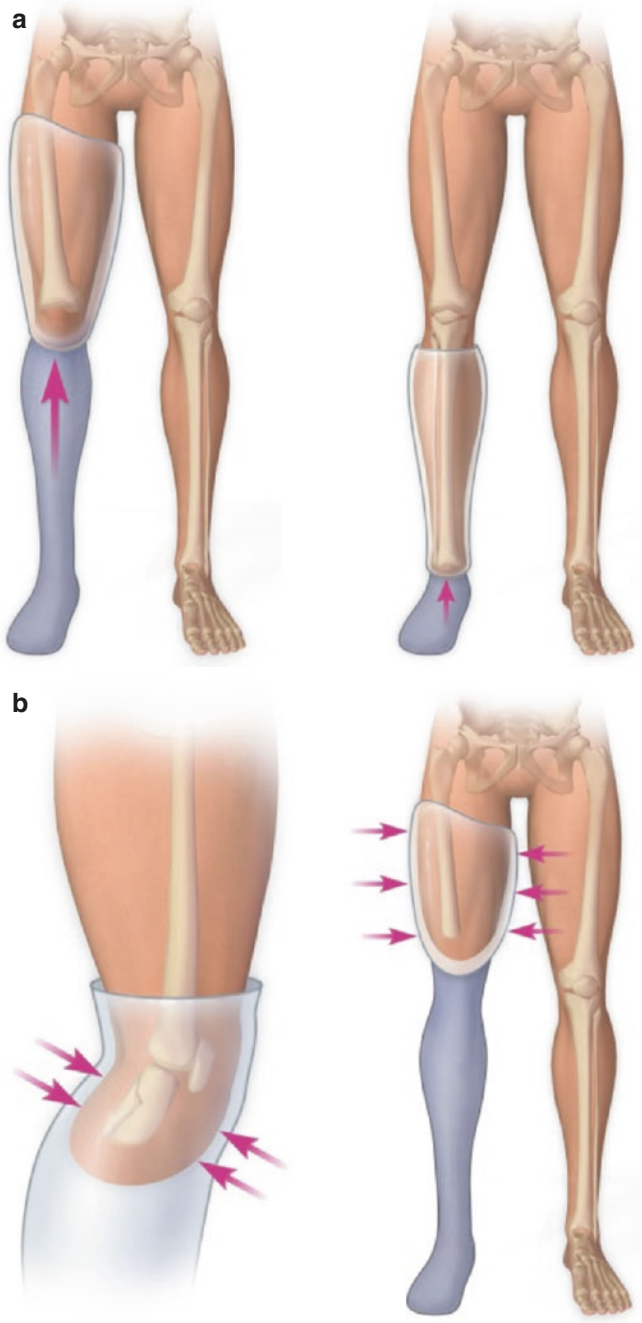

Fig. 23.3 (**a**) Direct load transfer (endbearing) is accomplished in knee disarticulation and Syme's ankle disarticulation amputation levels. (**b**) Indirect load transfer (total contact) is accomplished in transtibial and transfemoral amputation levels

amputation levels, where the surface area and stiffness of the terminal residual limb require unloading. The weight-bearing load must be applied to the entire surface area, with the soft tissue envelope acting as a cushion [14] (Fig. 23.3).

Soft Tissue Envelope

The soft tissue envelope acts as the interface between the bone of the residual limb and the footwear or prosthetic socket. It functions both to cushion the underlying bone and dissipate the pressures and forces applied during weight bearing. Ideally, it should be composed of a mobile, non-adherent muscle mass, and full-thickness skin. If the soft tissue envelope is adherent to bone, the shear forces will produce skin blistering, ulceration, and tissue breakdown. The residual limb should be durable enough to tolerate the direct pressures and pistoning within the prosthetic socket.

Healing Parameters

Vascular Perfusion

Amputation wounds generally heal by collateral flow, so arteriography is rarely a useful diagnostic tool to predict wound healing. Doppler ultrasound has been utilized to assess blood flow in the extremity before amputation. An ankle-brachial index of 0.45 in the patient with diabetes has been considered adequate for healing as long as the systolic pressure at the ankle was 70 mmHg or higher. These values are falsely elevated, and non-predictive, in at least 15% of patients with peripheral vascular disease because of non-compressibility of calcified peripheral arteries [15]. This has prompted the use of varying noninvasive vascular testing modalities, including transcutaneous partial pressure of oxygen ($TcPO_2$), skin perfusion pressure (SPP), and toe brachial index (TBI) [16]. The Vascular laboratory can measure toe pressures as an indicator of arterial inflow to the foot. This is owing to the observation that arteries of the hallux do not seem to be calcified, as do the vessels of the leg [17–20]. The accepted threshold toe pressure is 30 mmHg. Vascular consultation should be obtained for patients who do not have adequate vascular inflow on these exams.

Nutrition and Immunocompetence

Preoperative review of nutritional status is obtained by measuring the serum albumin and the total lymphocyte count (TLC). The serum albumin should be at least 3.0 gm/dL and the total lymphocyte count should be greater than 1500. The TLC is calculated by multiplying the white blood cell count by the percent of lymphocytes in the differential. When these values are suboptimal, nutritional consultation is helpful before definitive amputation. If possible, surgery in patients with malnutrition or immunodeficiency should be delayed until these issues can adequately be addressed. When infection or gangrene dictate urgent surgery, surgical debridement of infection, or open amputation at the most distal viable level can be accomplished until wound healing potential can be optimized [21–24]. At times, such as with severe renal disease, the nutritional values will remain suboptimal and distal salvage attempts may still be pursued, but at known higher risk for failure.

Poor glycemic control has been identified as a risk factor associated with a higher frequency of amputation (Fig. 23.4)

Comparison between the major amputation and minor or non-amputation groups in each item

Item	Subgroup	Number (n = 210)	Major Amputation Group	Minor Amputation Group and non-amputation group		x^{2a}	*p* value
				Minor Amputation Group (n = 65)	Non-amputation group (n = 100)		
Age at the initial examination[b]	A ≥ 65 years	115	32	37	46	0.002	0.964
	B < 65 years	95	13	28	54		
Sex	A Male	113	37	35	41	2.443	
	B Female	97	8	30	59		0.118
Duration of	A ≥ 18.1 years	96	20	37	39	0.496	
Diabetes[b]	B < 18.1 years	112	25	28	59		0.481
HbAl c	A ≥ 8.0%	94	33	36	25	4.409	
	B < 8.0%	116	12	29	75		0.035 *
Neurological Symptoms[c]	A Yes	165	43	58	64	0.283	
	B No	38	0	7	31		0.595
Retinal Symptoms[c]	A Yes	165	43	58	64	0.139	
	B No	39	1	7	31		0.709
Renal Symptoms[c]	A Yes (with dialysis)	61	30	18	13	7.875	0.0051 *
	B Yes (without dialysis)	96	11	37	48	0.336	0.562
	C No	54	4	10	39		
Dialysis[b,c]	A ≥ 6 years	38	22	9	7	3.379	0.053 *
	B< 6 years	22	7	9	6		
ASO (number of cases)[c]	A Yes (with multiple lesions)	71	39	20	12	10.1	0.0015 *
	B Yes (without multiple lesions)	79	5	37	37	1.918	0.1661
	C No	47	1	8	38		
Ishemic Heart Disease	Yes	64	28	21	15	2.517	
	No	143	17	44	82		0.1126

[a]Difference in the amputation rates determined by the log-rank test between subgroups fromed by Kaplan-Meier method
[b]Subgroups classified by median value
[c]Some cases are unclear or lacking data
* Significant difference

Fig. 23.4 Glycemic table and higher frequency of amputation

[25, 26]. High glucose levels will deactivate macrophages and lymphocytes and may impair wound healing. Additionally, high perioperative glucose levels are associated with systemic postoperative infections including those of the urinary tract and respiratory system. Ideal management involves maintenance of glucose levels below 200 Mg/dL. Elevation of the HgA1C above 8% has been associated with slower wound healing [24]. Caution must be taken in managing the perioperative patient's glucose with calorie reduction as this process may lead to significant protein depletion and subsequent wound failure. If the patient's BMI is normal, 25 cal/kg is a necessity to provide maintenance and to avoid a negative nitrogen balance.

The combined wound healing parameters of vascular inflow and nutritional status have been studied and shown to significantly affect healing rates for pedal amputations. Attempting to optimize nutrition and perfusion preoperatively, when medically possible, will limit the risk of wound complications and failure.

Perioperative Considerations

Pedal amputations may be performed under local or regional anesthesia. The effectiveness of local anesthetics may be impaired by the presence of infection and may need to be administered proximal to any cellulitis. When amputating above the ankle, spinal or general anesthesia will be necessary. Spinal anesthesia is contraindicated in the patient with sepsis demonstrated by fever over 100 °F or positive blood cultures.

Culture-specific antibiotic therapy should be continued perioperatively. If the focus of infection is completely removed with amputation, then the antibiotics may be discontinued 24 h after surgery. If, however, infection remains a concern, then antibiotics are continued for a soft tissue course of 10–14 days, or 6–8 weeks for bone infection.

Tourniquets may be needed to control bleeding during surgery. The surgeon must ensure that the tourniquet is not placed over a vascular anastomosis site or distal to an area of infection. The patient with severe vascular compromise will not require a tourniquet.

Preoperative Summary

Preoperative planning for distal limb salvage procedures should include the measurement of serum albumin, TLC, and tissue perfusion. With satisfactory values in all three categories, healing rates as high as 90% may be attainable. However, at least 10% of optimized patients may experience wound failure. With impaired nutrition or perfusion the risk of failure becomes even greater. The patient should be informed of these risks. Efforts should be made to use this information to plan procedures at levels that will limit the patient's exposure to multiple revision attempts. A single surgical session for a more proximal amputation may be preferable to multiple attempts at distal salvage in severely compromised or borderline cases.

Toe and Ray Amputations

Indications

Single toe amputation or ray resection may be performed for irreversible necrosis of a toe without medial or lateral extension. Deep infection of an ulcer to bone is also an appropriate indication for toe amputation. If uncontrollable infection extends to the metatarsal-phalangeal joint or metatarsal head, ray resection is appropriate. This procedure is also useful for infection or necrosis of the toe, requiring more proximal resection to obtain viable wound margins.

Ray resection is an excellent method of decompressing deep fascial infection limited to one compartment of the plantar structures of the foot, be that medial, lateral, or central. In such cases the wound is always left open to allow continued drainage and resolution of the acute infection. These wounds may be left open and allowed to granulate and heal by secondary intention. The surgeon may also choose to perform a delayed primary closure once the patient has been optimized. The other option is conversion of the limb to a more proximal, definitive amputation [27]. With isolated or multiple ray amputations, there is an elevated risk for transfer ulceration development which may require other metatarsal head resections, Achilles/gastroc lengthening, or more proximal amputation.

Procedure

First and fifth ray amputations are a wedge resection of the digit and the incision converges along the medial or lateral aspect of the metatarsal, respectively.

Central ray incisions are different from those of the first and fifth rays. Incisions are made on the medial and lateral aspect of the base of the digit and extend proximally on the dorsal and plantar aspect of the foot to converge over the individual metatarsal. If ulceration is present, as frequently occurs plantar to the metatarsal head, the ulcer is excised along with the wedge of soft tissue that includes the affected toe. The initial incisions are carried to bone, and the toe is disarticulated at the metatarsal-phalangeal joint. The periosteum of the metatarsal is reflected as far proximally down the shaft of the bone in order to assure that the resection is performed at a level of viable, noninfected bone. The bone is usually cut at

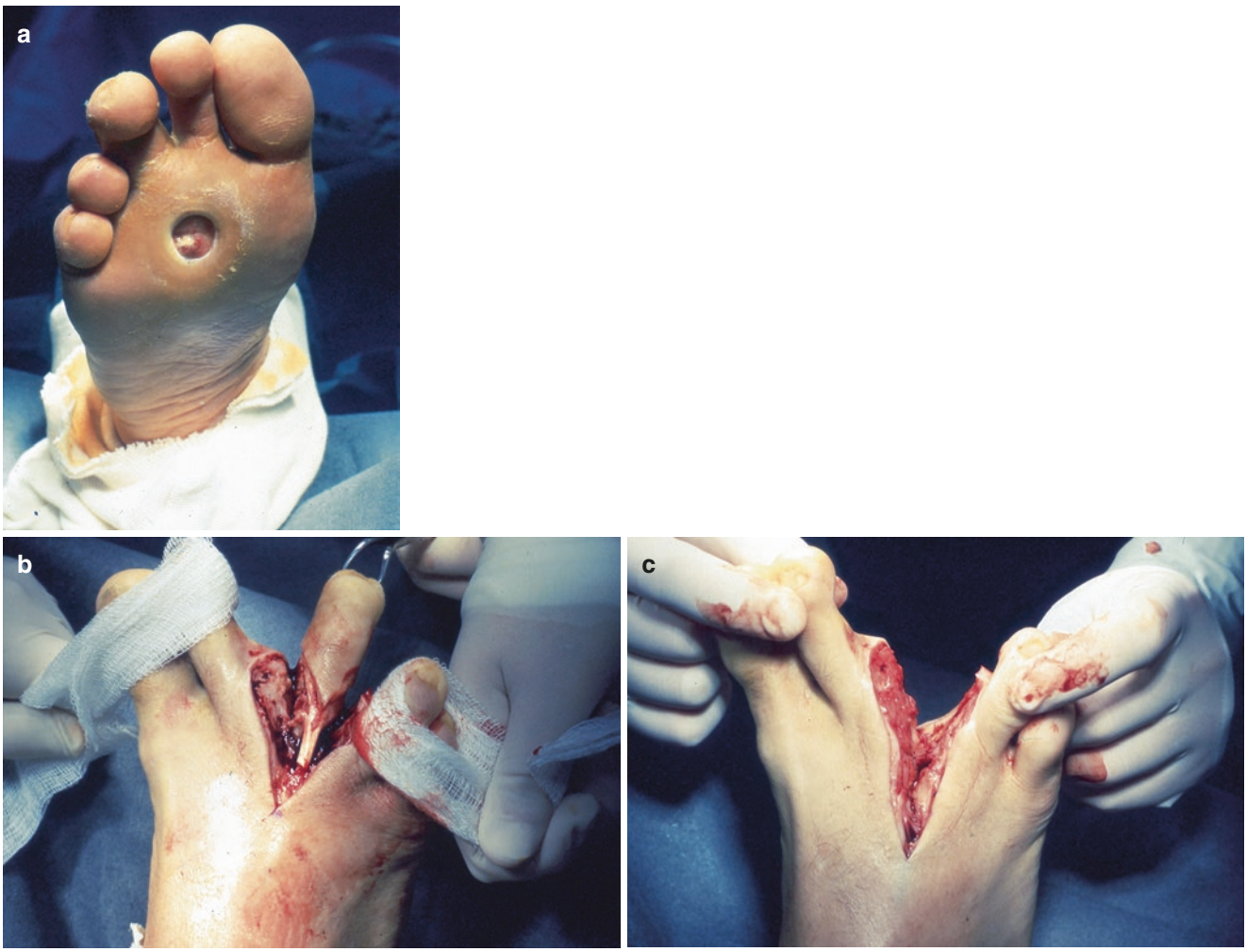

Fig. 23.5 (**a**) Plantar third metatarsal head ulcer. (**b**) Dorsal skin incision. (**c**) Patient after third ray amputation. Note that the plantar ulcer was also excised

the proximal diaphysis, or diaphyseal-metaphyseal junction. It is rarely necessary to do the extensive dissection required to disarticulate the metatarsal cuneiform joint.

Once the bone is removed from the wound, the foot is compressed from proximal to distal to assure that there is no remaining ascending purulent drainage. If the flexor or extensor compartments reveal purulence on compression, then they are incised and irrigated to decompress any remaining infection. If the ray resection was performed for metatarsal or plantar space infection, it is left open to allow healing by secondary intention or converted to a delayed primary closure (Fig. 23.5).

Postoperative Care

The only ray resection that should be closed primarily is one performed for infection localized to the toe, with clearly viable wound edges, and no suggestion of proximal infection. In this case a gauze dressing is applied and the patient is maintained in a postoperative shoe until healed. A cane or walker is utilized for protected weight bearing.

In cases where the wound is left open, culture-directed antibiotic therapy should be administered for soft tissue or bone infection depending on the extent of the infection. Infectious Disease service consultation is advisable. The open wound should be treated according to the surgeon's preferred protocol. If there is significant depth and/or drainage of the wound, the surgeon may contemplate the use of alginates or a negative pressure system. Packing should be sufficient to absorb excess drainage, but not aggressive enough to interfere with wound contraction. The foot should be protected from full weight bearing during this time with the appropriate gait assistive device. A physical therapy consult may be required to assure appropriate gait training and/or use of gait assistive devices.

Once healing has been achieved, the patient should have a prescription for protective foot gear. If there is evidence of

pressure keratosis developing adjacent to the ray resection site, the patient should be seen in clinic as necessary to pare the callus in order to prevent transfer ulceration.

Complications

Persisting infection is rare if the wound was adequately debrided at the time of the ray resection. However, if residual infection is suspected, follow-up surgical debridement should be performed. Wound failures can result from inadequate healing parameters, such as impaired blood flow, or abnormal serum albumin. Such metabolic wound failures may require more proximal amputation to obtain healing.

The most common late complication of ray resection is transfer lesion and re-ulceration. If pressure keratosis or ulcerations cannot be managed with debridement and prescription shoes, consideration is given to gastrocnemius recession or Achilles lengthening if ankle equinus exists. The author's internal study of such procedures for forefoot ulcerations shows that these procedures can produce remission of 90% of forefoot ulcers for at least 2 years. If this approach fails, resection of the remaining metatarsal heads, or more proximal amputation may become necessary [28].

Transmetatarsal and Lis Franc Amputation

Indications

McKittrick advocated the transmetatarsal amputation in 1949 [29] for infection or gangrene of the toes in diabetic patients. Wagner, in 1977, subsequently recommended this amputation for use in patients with diabetic foot complications [30], advocating preoperative vascular review. He advised that Doppler studies demonstrating an ankle-brachial artery index greater than 0.45 could predict healing of the procedure with 90% accuracy. The authors' group reviewed 64 transmetatarsal and Lis Franc amputations in 1986 [31]. These amputations were performed for gangrene of the forefoot, or forefoot ulcers recalcitrant to nonsurgical attempts at healing. Their results indicated that patients with Doppler ankle-brachial artery index above 0.5 combined with serum albumin levels greater the 3.0 gm/dL and TLC greater than 1500/cm^3 healed at a rate of 92%. Those patients lacking one or more of these three indicators healed at a rate of 38%.

As stated earlier, amputation of a single toe or metatarsal may be successfully performed for patients with a localized ulceration if preoperative healing indices are satisfactory. However, even if early healing is achieved, there can be significant transfer ulceration following such procedures leading to later complications [27].

This experience suggests that transmetatarsal amputation may be a more definitive procedure for the management of forefoot ulceration. Transmetatarsal amputation may be considered for patients with more than one ulceration or site of necrosis of the forefoot. Likewise this procedure may be considered in cases with a significant nonhealing ulceration or foot deformity that is likely to lead to subsequent ulcer. However, transmetatarsal amputation does not assure that no further ulceration of the foot is likely.

In our long-term review of midfoot amputations, including transmetatarsal and Lis Franc procedures, 9 out of 64 feet sustained new ulcerations within the first year after healing the primary procedure [32]. The source of these ulcerations included hypertrophic new bone formation, and subsequent varus or equinus deformity. These dynamic deformities occurred more in Lis Franc amputations, where muscle imbalance was more likely to occur because of the loss of the attachments of the peroneal and extensor tendons.

Plantar ulceration under the metatarsals may deter the surgeon from a transmetatarsal amputation because of the inability to preserve a long plantar flap for wound closure. Sanders determined that a V-shaped excision of the ulceration, with the apex proximal, and the base at the junction of the dorsal and plantar flaps, allows conversion of the wound to a T-shaped closure (Fig. 23.6) [33]. Salvage of the plantar flap allows the surgeon to perform a transmetatarsal amputation rather than requiring a more proximal Lis Franc operation to eliminate the plantar ulcer. The specific indications for transmetatarsal amputation remain similar to McKittrick's: chronic ulceration or gangrene of the toes. These procedures are likely to heal when albumin, TLC (total lymphocyte count), and arterial inflow meet recognized minimal standards. Before a definitive midfoot amputation, acute infection should be stabilized by incision and drainage, debridement, or ray resection. Infected tissue present at the time of the definitive procedure can be expected to compromise success and should be eliminated in a staged procedure, if necessary. If these criteria cannot be met, then proximal amputation may be more appropriate.

Technique

This procedure can be performed with monitored anesthesia care, regional or ankle block. General anesthesia is rarely necessary and spinal is avoided if any concern for sepsis is noted. Appropriate medical clearance should be obtained regarding glycemic management and cardiovascular risks.

The transmetatarsal and Lis Franc amputations differ in technique mainly at the point of amputation of the forefoot from the hind foot. The transmetatarsal procedure is performed through the metatarsal bases, leaving the insertion of tibialis anterior, peroneus longus, and peroneus brevis intact.

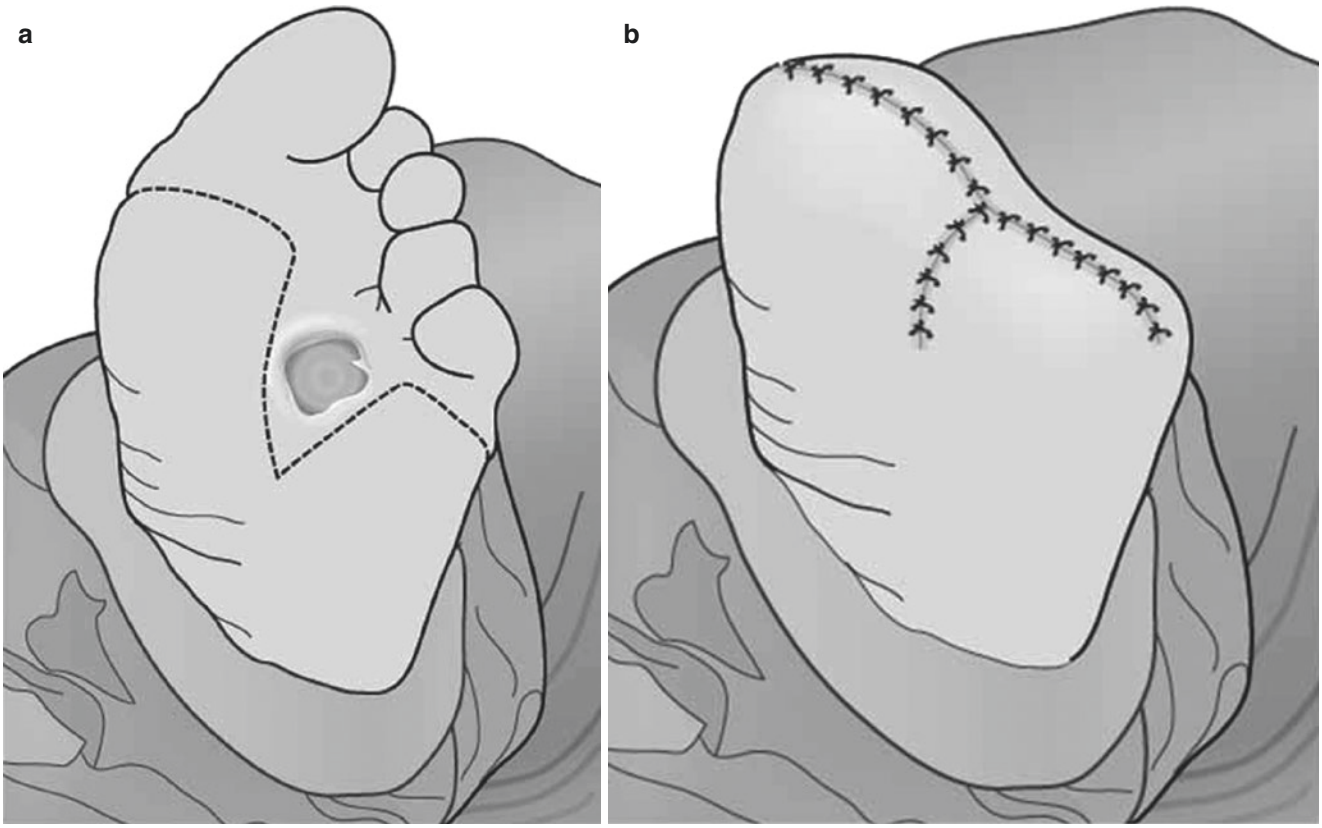

Fig. 23.6 (**a**) The Sander's technique for plantar flap revision with transmetatarsal amputation in the presence of a distal plantar ulcer. (**b**) The margins of the ulcer site are then approximated with closure as shown. Image courtesy of Lee Sanders, DPM

The metatarsal osteotomy should be performed through the proximal metaphysis in order to achieve adequate soft tissue coverage of the residual limb. The Lis Franc amputation requires disarticulation at the metatarsal cuneiform and cuboid joints, resulting in loss of the tendon insertions mentioned previously. The authors have made occasional attempts to preserve the base of the fifth metatarsal and peroneus brevis insertion, but this is not always practical or beneficial.

The procedure begins with a dorsal incision across the metatarsal bases, from the medial to the lateral side of the foot, deferring the plantar incision for the time being. The incision is carried to bone through the dorsal tendons and neurovascular structures. Arteries are identified and ligated. The periosteum of the metatarsal bases is incised and reflected using an elevator to expose either the site of the intended osteotomy, or the metatarsal tarsal articulation.

If a transmetatarsal amputation is to be performed, the osteotomies are now initiated. Using a power saw the first metatarsal is cut, directing the blade slightly medial and plantar. The second, third, and forth metatarsals are cut, taking care to produce an anatomic parabola, leaving no residual metatarsal particularly longer than the adjacent bone. The fifth metatarsal is cut last, directing the blade slightly lateral and plantar. At this point the plantar incision is made, initiated at a 90° or less angle to the dorsal incision, carried distally to the sulcus, around the metatarsal heads, and then posteriorly along the lateral side of the foot to the fifth metatarsal base. The incision should be carried to bone as much as possible. If plantar metatarsal head ulceration is present, it should be excised using a V-shaped wedge, directing the apex proximally, and the base distally at the level of the distal transverse incision. When this is closed, it results in a T-shaped flap.

The metatarsals may now be dissected from the plantar flap from proximal to distal, dissecting along the metatarsal shafts in order to preserve as much of the soft tissue structures in the plantar flap as possible. The remaining distal attachments of the metatarsal heads are cut, and the forefoot is amputated. Significant vascular structures should be ligated. The entire wound should be thoroughly irrigated. Remaining fibrous, ligamentous, and exposed tendinous structures should be cleanly cut from the flap. Minimal debulking of remaining intrinsic muscle structures may be performed if necessary to obtain approximation of the dorsal and plantar flaps (Fig. 23.7). The wound is closed in two to three layers, starting with sutures placed in the middle of the plantar flap musculature, and approximated to the intermetatarsal or intertarsal ligamentous

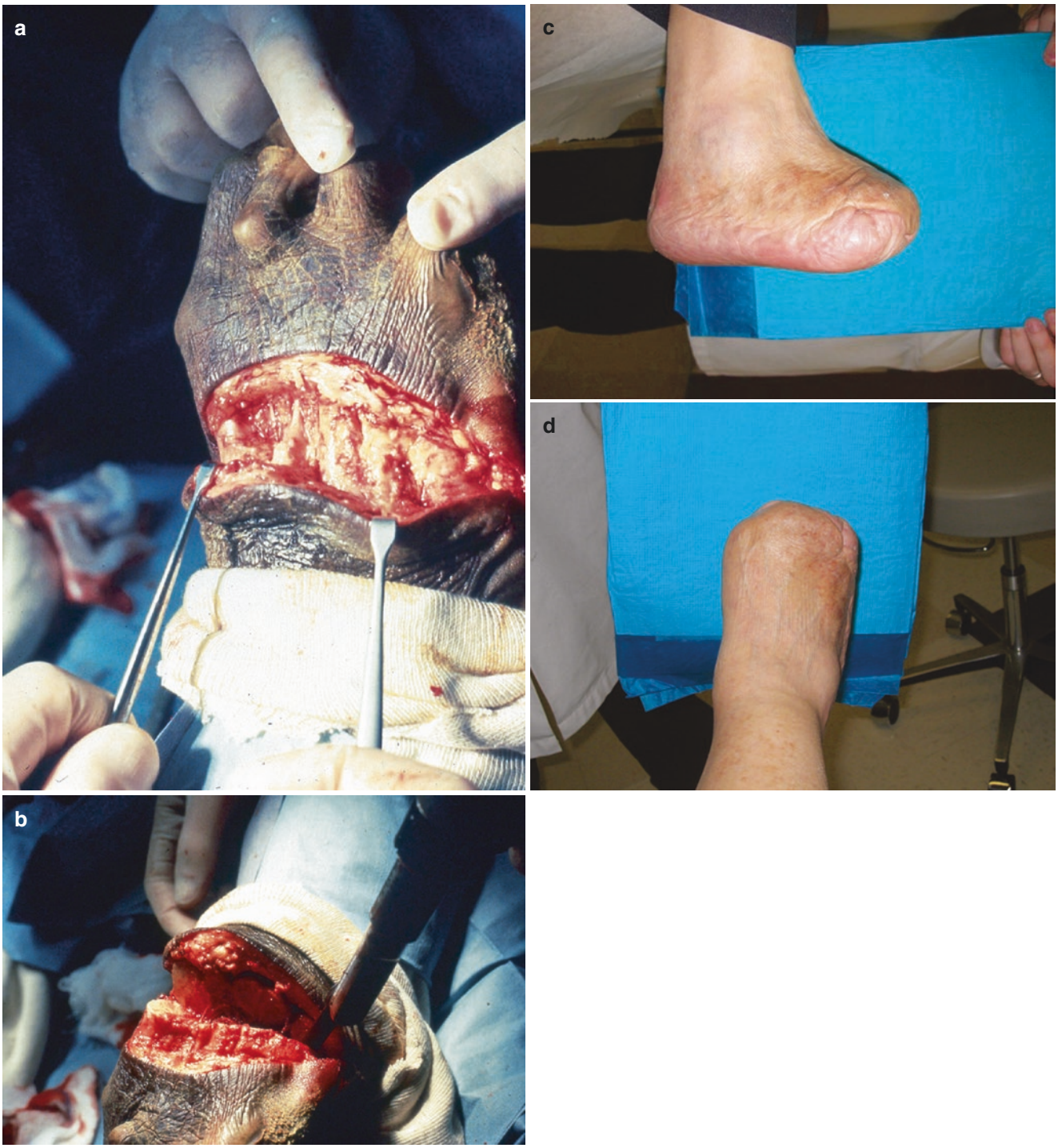

Fig. 23.7 (**a**) Dorsal incision with exposure of metatarsal. (**b**) Proximal metatarsal osteotomies to provide sufficient soft tissue coverage. (**c**, **d**) Healed transmetatarsal amputation without equinus, lateral and DP view

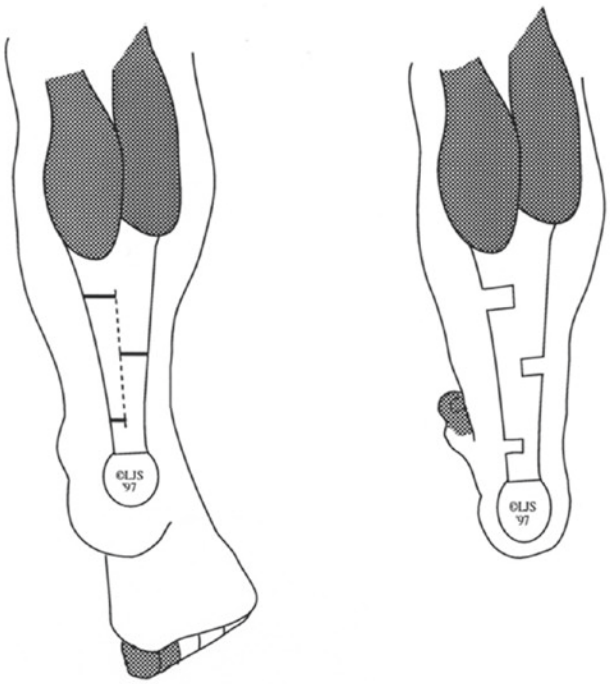

Fig. 23.8 A percutaneous Achilles lengthening as described by Sanders. Image courtesy of Lee Sanders, DPM

structures. Then subcutaneous sutures are passed from the distal deeper layers of the flap to the dorsal retinaculum. The skin is closed with staples or mattress/simple interrupted sutures of 3-0 nylon as needed to obtain a satisfactory incision line.

The technique is similar for a Lis Franc amputation, except that the metatarsal cuneiform and cuboid articulations are detached instead of the aforementioned metatarsal osteotomies. The first cuneiform is invariably long, and needs to be rongeured or cut proximally to balance the parabola with the remaining metatarsals. This cut should be directed slightly medially and plantarly. Articular cartilage from the remaining tarsals is rongeured to bleeding cancellous bone. Since adapting Sanders' plantar flap technique, the authors perform very few Lis Franc procedures because of the functional disadvantage of varus and equinus associated with this procedure. If a Lis Franc is the only option, tibialis anterior is released from the medial side of the first cuneiform, and a percutaneous tendo-Achilles lengthening is performed (Fig. 23.8).

Prior to closure, the wound should be thoroughly irrigated. If a tourniquet was used it should be released, and significant hemorrhaging vessels ligated. Because the procedure leaves relatively little dead space, drains are rarely necessary. The surgical site is closed similar to the transmetatarsal amputation described above.

Postoperative Care

Mild compression and protection of flap from tension are the authors' objectives in immediate postoperative wound care. In order to accomplish this, a soft gauze roll dressing is applied from the foot to the ankle. Moderated compression is applied, with minimal force directed from plantar to dorsal in order to protect the plantar flap from undue stress on the incision line. Two to three layers of cast padding are applied from the foot to the tibial tuberosity, maintaining the foot and ankle in neutral position, neither dorsiflexed or plantar flexed. Finally, several layers of 5 × 30 in. plaster of Paris splints are applied posteriorly from the tip of the residual foot to the calf, distal to the knee. The splints are wrapped with another two layers of cast padding, and an ace wrap secures the entire dressing. This resembles a Jones dressing, protecting the wound from any contusions, and from any dorsal or plantar tension.

This dressing is left in place for approximately 48 h before the surgical site is inspected. A similar dressing is maintained for 2–4 weeks until the incision line is clearly stable. During this time the patient is instructed in the use of crutches, a walker, or wheel chair with leg elevation. Minimal heel touch weight bearing on the operated foot is allowed until the wound is clearly stable, and free of the risk of major dehiscence. Occasional superficial dehiscence may occur, especially in high-risk patients. This is treated like any other grade I ulcer with cleansing, debridement, and topical wound care measures until healed. Major postoperative dehiscence, infection, or necrosis of the plantar flap will likely require revision surgery.

Complications

Wagner has stated that distal amputations can be expected to heal up to 90% of the time in diabetics who exhibit adequate circulation as determined by Doppler examination demonstrating ankle-brachial artery index of 0.45 or better [30]. The authors' group confirmed that healing could be achieved in over 90% of patients with diabetes undergoing midfoot amputation if ankle-brachial artery index is over 0.5, serum albumin is greater than 3.0 gm/dL, and TLC is over 1500/cm^3 [31]. However, we have also noted that up to 42% of midfoot amputations may suffer some form of complication, even though the majority may ultimately heal their surgical wounds [32]. The complications include early wound dehiscence, and late re-ulceration, which can be treated successfully to result in limb salvage in most cases. Patients most likely to suffer wound dehiscence include those with marginal vascular indices and low serum albumin. This is especially true in renal failure patients. These prognostic indicators should be taken into consideration in preoperative planning and discussed with the patient. Those

at high risk for failure may be better served by a higher amputation more likely to heal with one operation.

Biomechanical abnormality resulting from muscle imbalance can result in dynamic varus, producing lateral foot ulceration. This is particularly true in Lis Franc amputations because of the varus pull of an unopposed tibialis anterior. Tibialis anterior tendon transfer in some cases can successfully treat this. Armstrong and associates [34] noted that bone regrowth after partial metatarsal amputation resulted in a significantly increased risk of re-ulceration. This regrowth was likely to occur in metaphyseal procedures, in males, when manual bone cutting equipment was utilized. In our experience, these re-ulcerations can be treated with aggressive ostectomy of the underlying bone and standard subsequent wound care.

Chopart Amputation

Indications

Francoise Chopart described disarticulation through the midtarsal joint while working at the Charitable Hospital in Paris in the 1800s [35]. The operation has been thought to have limited applications because the residual foot is susceptible to progressive equinovarus deformity. The Chopart amputation is gaining new favor because the length of the limb is retained and the potential complications of the procedure can be successfully addressed. Combining ankle fusion with hindfoot amputation allows apropulsive ambulation with a modified high-topped shoe [35–38].

Amputation levels are usually chosen on the basis of tissue viability and residual limb function. A Chopart level amputation may be considered when the longer transmetatarsal or Lisfranc amputation level is not an option because of the extent of forefoot tissue necrosis. Half of all patients undergoing an initial nontraumatic amputation will likely require an amputation of the contralateral limb [39]. As discussed earlier there is a higher metabolic requirement for ambulation in those patients who undergo more proximal amputations. The decision on amputation level should attempt to maximize the patient's mobility and independence by preserving length whenever possible, thus making the Chopart amputation useful in cases where more distal foot procedures are not feasible.

An open Chopart amputation is useful when stabilizing a grossly infected forefoot. This may then be converted to a Boyd or Syme's amputation when soft tissue infection is resolved. The open Chopart amputation procedure disarticulates the foot at the level of the calcaneocuboid and talonavicular joints, leaving the articular surfaces intact. The proximal spread of infection may be less likely with the cancellous spaces unopened [40]. During the open Chopart procedure, care must be taken to visualize and resect all necrotic and/or nonviable tissue. Compression of the limb proximal to the open amputation site is done manually to identify purulent drainage from the compartments of the leg. If purulence is expressed with compression, then the affected compartment must be incised and irrigated to provide adequate drainage. Once the acute infection is resolved and the healing parameter indices are suggestive of healing, the open Chopart may be revised to a definitive amputation. If the surgeon anticipates that the acute infection may be stabilized, and healing is anticipated at the Chopart level, then care must be taken to retain sufficient soft tissue to provide coverage of the residual foot.

The prerequisite for a definitive Chopart amputation is that the plantar heel pad and ankle/subtalar joint articulations are not compromised [41]. A definitive Chopart amputation is considered if the forefoot infection extends proximal to the metatarsal bases and neither a transmetatarsal nor a Lisfranc amputation can be salvaged. Reyzelman et al. [42] suggest that a Chopart amputation is more advantageous than a short transmetatarsal or a Lisfranc amputation because it does not disrupt the transverse arch of the foot. The disruption of the transverse arch creates an overpowering of the tibialis anterior, tibialis posterior, and gastrocnemius muscle to the peroneus brevis muscle. The muscle imbalance created in the short transmetatarsal or Lisfranc amputation may lead to a varus rotation of the residual foot. A frontal plane rotation of the weight-bearing surface of a Chopart amputation is less likely to occur, unless the calcaneus or ankle is structurally in varus [43]. The Chopart amputation does, however, lead to an equinus deformity because of the unopposed pull of the Achilles tendon. An Achilles lengthening and/or performing a tibialis anterior transfer at the time of the definitive closure may address this.

Bowker [44] and Marquardt [45] developed methods to maintain motor balance and limit the equinus related to this amputation level. A two-centimeter section of Achilles tendon is resected. During the procedure, vertical slots are created in the talar head for the tibialis anterior tendon and the extensor halluces longus tendon and in the anterior calcaneus for the extensor digitorum longus tendon. The plantar margin of the calcaneus is rongeured smooth. Each of the tendons is anchored to the plantar capsule or central plantar fascia to stabilize the respective tendons in the grooves.

Some authors suggest arthrodesis of the subtalar and ankle joints to improve stability and remove the risk of equinus. These recommendations must be weighed by considering the likelihood of successful arthrodesis and the healing of additional incisions in an already compromised patient.

Technique

If the Achilles tendon is to be lengthened/resected, this is done first, prior to uncovering the foot. The Achilles wound is sutured and dressed. Then, attention is directed to the foot. The dorsal incision begins from the tuberosity of the navicular extending dorsolateral to the mid cuboid level. The medial and lateral incisions are carried distally to the

mid shaft level of the first and fifth metatarsal and continued transversely at this level along the plantar aspect of the foot. These incisions form a "fishmouth" creating dorsal and plantar flaps. The incisions are deepened to expose the talonavicular and calcaneocuboid joints. The tibialis anterior, EHL, and EDL should be identified and preserved for later transfer to the talar and calcaneal necks. The remaining soft tissue structures are incised to complete the disarticulation of the forefoot from the rearfoot. The articular cartilage of the talus and calcaneus should be resected creating a flush surface when the definitive procedure is being performed. The tibialis anterior, EHL, and EDL tendons are anchored through channels in the talus and calcaneal neck to the plantar capsule or fascia. If a tourniquet has been utilized, it is deflated and hemostasis is achieved. The surgical site is closed as described previously. A drain is necessary only if there is significant dead space, or if excessive bleeding is anticipated, to prevent hematoma formation. A sterile compressive dressing and a posterior splint are applied to the lower extremity.

Postoperative Care

The patient is maintained non-weight bearing in a posterior splint or below knee cast until the wound is healed for up to 6 weeks if necessary. The Chopart amputee without equinus is capable of ambulating in an inlay-depth high top shoe with a forefoot filler but functions best with a polypropylene solid AFO prosthesis with a foam filler [40]. The prosthesis helps to eliminate or minimize the pistoning motion of the distal amputation in a conventional shoe. If the Chopart amputee has an equinus (and is not a surgical candidate for TAL), then they should be fitted for a clamshell prosthesis (Fig. 23.9) [46].

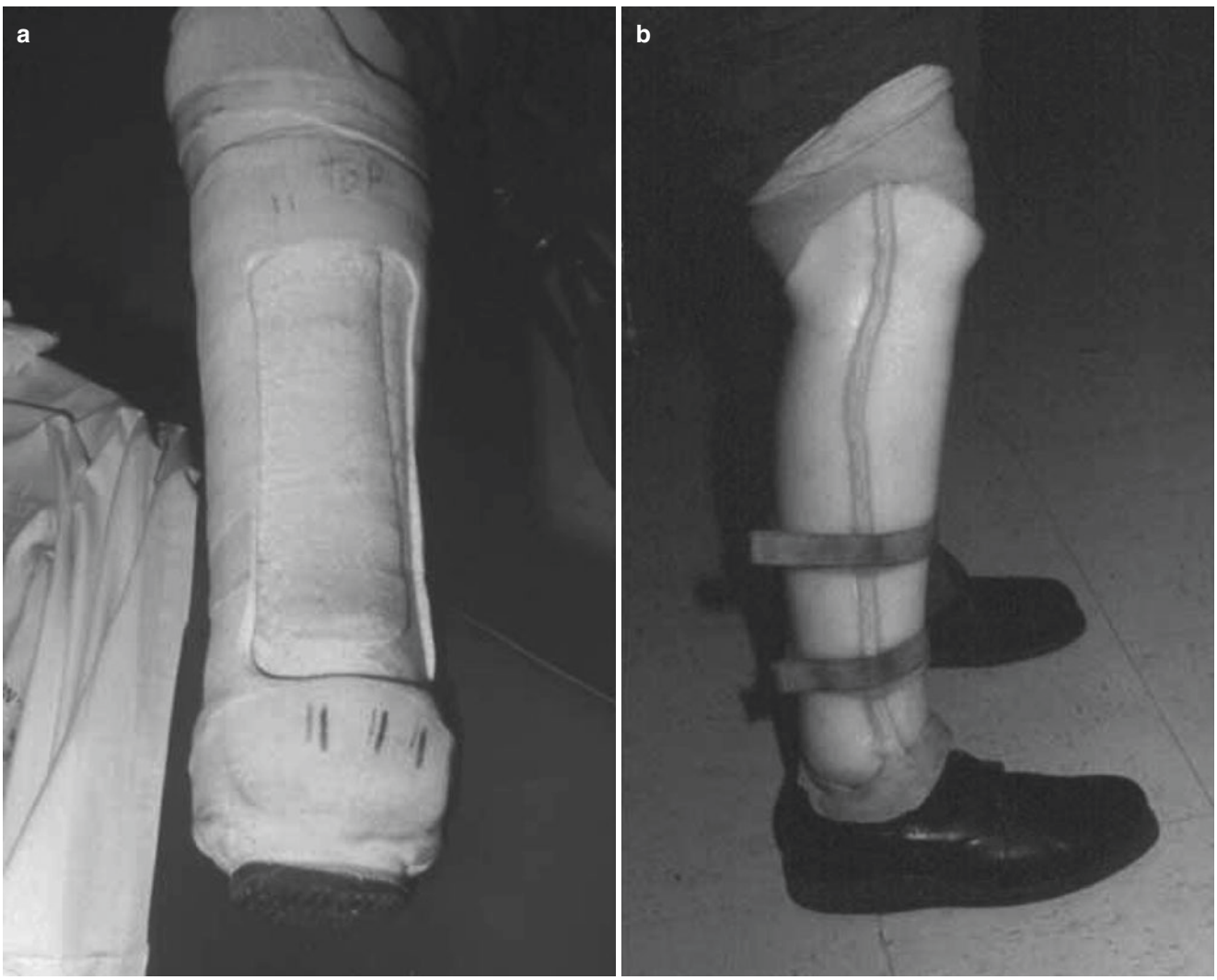

Fig. 23.9 (**a**) A fiberglass cast with a distal rubber bumper and a medial window is used as a temporary prosthesis to allow early ambulation for the Syme's amputation patients. (**b**) A thermoplastic variation of a temporary prosthesis with a prosthetic foot attached. In a patient with very limited ambulation, this may also serve as permanent prosthesis. (**c**) A variety of Chopart prostheses have been advocated. This prosthesis has a posterior closure

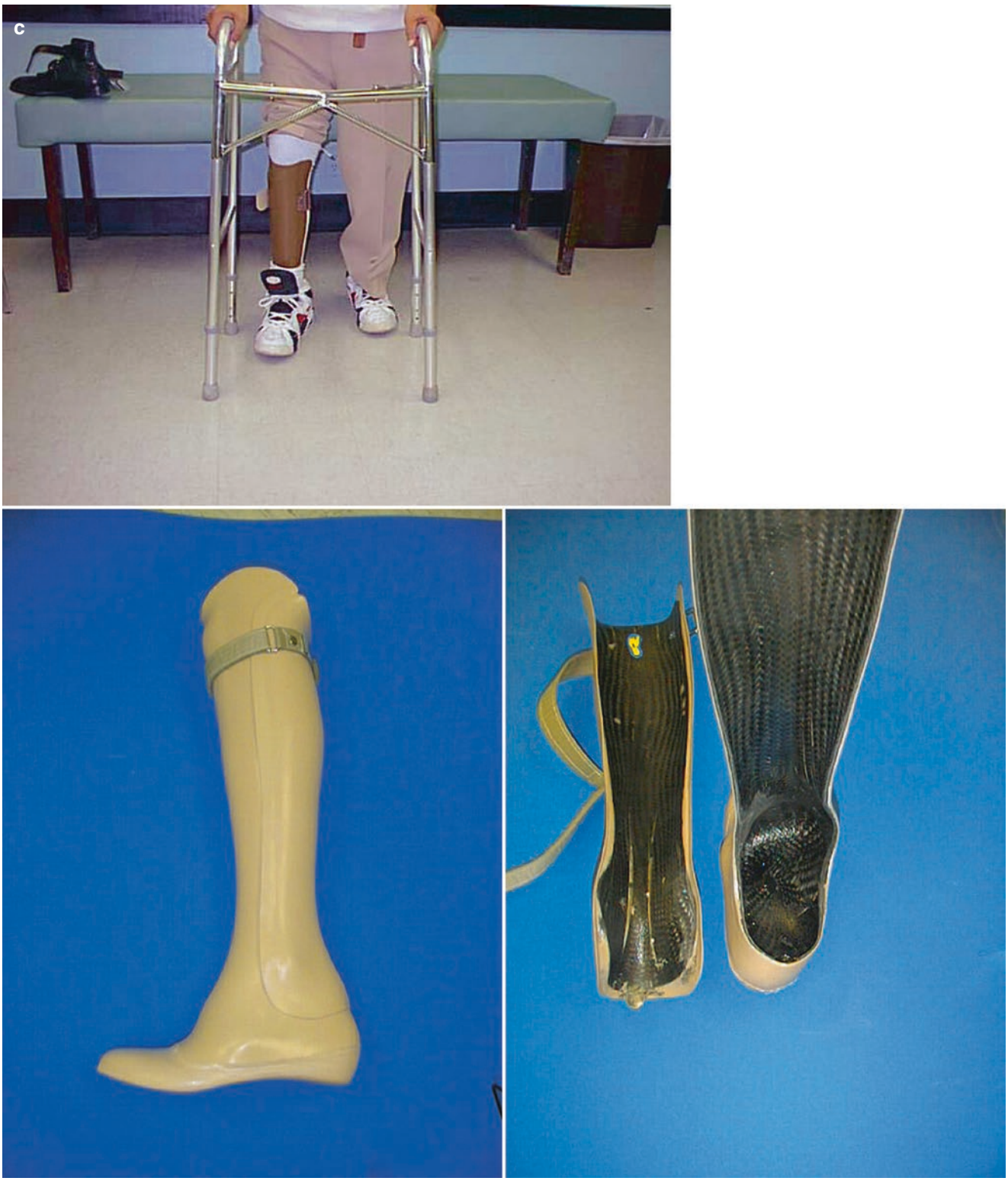

Fig. 23.9 (continued)

Complications

Infection or wound failure are not complications specific to the Chopart amputation but is more likely if performed on patients who did not meet the generally accepted vascular and nutritional parameters described earlier. Care must be taken to fashion the flaps to provide adequate coverage for the residual foot without soft tissue being secured under excessive tension, as this may lead to wound dehiscence and/or devitalization. Equinus deformity can still occur even if Achilles lengthening is performed. The development of a plantar ulceration in a plantarflexed residual foot is a common occurrence and may lead to revision surgery. As always, close postoperative follow-up and early intervention may minimize these problems.

In spite of these shortcomings the Chopart amputation remains useful as an early incision and drainage procedure to stabilize acute infection. It is also useful as a definitive procedure in select cases because of its advantage of limb length and tissue preservation.

Long-Term Follow-Up Needs After Partial Foot Amputation

Patients with a history of ulceration remain at high risk for re-ulceration, even after the foot has been returned to grade 0 by a surgical procedure. The patient who has undergone any form of partial foot amputation should be placed in a high-risk foot clinic for regular follow-up visits. Both short- and long-term complications have been recognized. Even though the benefits of distal limb salvage are well accepted, biomechanical review and management visits must be included in aftercare for the amputation to be successful [47]. Early on, the residual limb should be protected with a posterior splint or cast and limited weight bearing. Rehabilitation should include crutch or walker training, if feasible. If the patient cannot use gait assistive devices, a wheel chair with leg lift, and instruction in wheel chair mobility and transfer techniques should be provided. These protective measures should be continued until wound is clearly healed.

Later, protective footwear, or even a plastizote-lined ankle foot orthosis may need to be prescribed for adequate protection. Although many patients may function well with an oxford shoe and anterior filler, others may need more elaborate orthotic management. Custom-made short shoes, rocker bottom shoes with a steel shank and anterior filler, or conventional shoes with an ankle foot orthosis have all been advocated. Each patient should be observed carefully as the return to full ambulation to determine the need for orthotic management. Computer-assisted pressure mapping may be helpful in determining the success of any device in off-loading residual pressure points. If keratotic lesions should develop, these should be considered pre-ulcerative and debrided regularly before ulceration can occur [48–50].

Transmetatarsal and Lis Franc amputation have the benefit of improved function and patient acceptance over higher amputation for individuals suffering from serious forefoot infection, ulceration, or gangrene. However, these operations must be recognized as high-risk procedures. Nevertheless, with appropriate preoperative planning, meticulous surgical technique, protective postoperative care, and long-term follow-up, midfoot amputations can be successful limb salvage techniques for most patients undergoing these procedures.

Transmalleolar Amputation: The Syme's Procedure

Indications

Hind foot amputation, to be successful, must produce a reliable result with a long-lasting and functional residual limb. Chopart's amputation at the talonavicular and calcaneal-cuboid joints creates significant muscle imbalance frequently resulting in ankle equinus and ulceration. The Boyd amputation has also been advocated [51]. This procedure involves fusion of a portion of the calcaneus to the distal tibia. The advantage is that the heel pad remains well anchored to the calcaneus. An additional problem becomes evident in attaining union of the tibia to calcaneus. There may also be difficulty in prosthetic fitting. The residual limb remains long and there is inadequate space to place a dynamic response prosthetic foot without raising the height of the contralateral limb to compensate for this addition. It is unknown whether this height difference results in gait problems for the diabetic patient.

The Syme's amputation is performed through the malleoli and results in physiologic load bearing throughout the residual limb. The fat pad takes load directly and transfers this directly to the distal tibia [52]. With the use of dynamic response feet, this amputation level results in decreased energy expenditure with ambulation compared to higher procedures or midfoot amputation [53–56]. Contraindications for this procedure include local infection or gangrene at the level of the amputation, and inadequate nutritional and vascular parameters to sustain distal healing. Healing may be achieved using this procedure with serum albumin levels as low as 2.5 gm/dL [52]. Heel ulceration has been considered a contraindication to a Syme's procedure in the past. However, an anterior flap may be useful in patients with a nonviable heel pad [55, 57]. A long-term review of this procedure modification in a significant series of patients has not yet been performed.

Procedure

The incision is placed anteriorly across the ankle mortise and then in a stirrup fashion across the anterior heel at the level of the malleoli. The incision is deepened at the anterior ankle and the ankle capsule is incised transversely. The ankle ligaments are released sharply and the talus is displaced anteriorly in the mortise. A bone hook is placed into the talus and used to anteriorly distract the talus so that soft tissues may be freed from the talus and the calcaneus. Care is exercised at the posterior calcaneus to prevent buttonholing of the skin while releasing the soft tissues. Once free, the residual foot is removed from the wound and the wound is thoroughly irrigated. The residual tendons are gently distracted 0.5–1 cm and sectioned. If needed the anterior ankle vessels may be ligated with appropriate suture. Anterior and posterior margins of the distal tibia may require debridement to diminish excessive spurring. Two drill holes may be placed in posterior tibia and/or the anterior tibia. A heavy absorbable suture (0) may be utilized through the drill holes to anchor the plantar fascia to the distal tibia. The anterior aspect of the residual plantar fascia is sutured into the anterior ankle capsule and the subcutaneous tissues and skin are closed in layers. A medium hemovac drain is placed prior to closure. A posterior splint or a short leg cast is placed. The drain is removed 24–48 h after surgery.

Postoperative Care

The patient may begin assisted/partial weight bearing at 3–5 days and is maintained in a short leg cast for 3–6 weeks. The patient is then advanced to a fiberglass cast temporary prosthesis with a rubber bumper distally. Once the patients' limb has matured and there is minimal residual edema, the patient is fitted for a Canadian Syme's prosthesis with a dynamic response foot (Fig. 23.10). Full activity is resumed. The need for physical therapy gait training is unusual.

Complications

Healing rates for this level vary from 70 to 80%. Early complications with the wound may occur in up to 50% of the patients. Most of these problems may be treated with local wound care, total contact casting, and culture-specific antibiotic therapy. Other problems include heel pad migration and new bone formation. Heel pad migration has become less frequent with anchoring of the fascia. Should new bone formation become significant or cause ulceration, ostectomy may become necessary [52].

Transtibial or Below Knee Amputation

Indications

Individuals with transtibial amputation provide the largest population of patients that are capable of achieving meaningful rehabilitation and functional independence following lower extremity amputation. The most predictable method of obtaining a durable residual limb is with a posterior myofasciocutaneous flap [58]. This level takes advantage of the plastic surgical tissue transfer technique of a composite tissue flap without dissection between layers, thus minimizing the risk for devascularization of the overlying skin.

Procedure

The optimal tibial transection level to optimize functional ambulation is a tibial length of 12–15 cm distal to the knee joint. The fibular amputation level in the past has been advised to be approximately 1 cm shorter than the tibia. In order to optimize the weight-bearing platform of the transtibial amputation stump, it is now felt that the fibula level should be just a few millimeters shorter than the tibia. The length of the posterior flap should be equal to the diameter of the limb at the level of the tibial transection level, plus 1 cm. A short "fishmouth" should be used on the anterior aspect of the stump to place the surgical scar in a better area for prosthetic fitting. The longitudinal component of the flap should range from one-third to one-half of the width of the limb, depending on the bulkiness of the leg. Thinner limbs with more tenuous blood supply are better performed with a width of approaching 50%, while the amputation stump in obese patients are best created with a width of approximately one-third the diameter (Fig. 23.11).

The anterior corner of the tibia should be beveled to decrease the shear forces on the anterior-distal aspect of the amputation stump. Historically, the posterior fascia of the gastrocnemius muscle has been sutured to the end of the anterior compartment fascia and the periosteum of the tibia. In order to create a better soft tissue envelope and enhance weight bearing, it is now advised to use a version of the "extended posterior flap" as described by Smith et al. [59]. In this method, the posterior gastrocnemius fascia is sutured to the anterior compartment of the leg and the periosteum of the tibia at a level of 1–2 cm proximal to the bony transection.

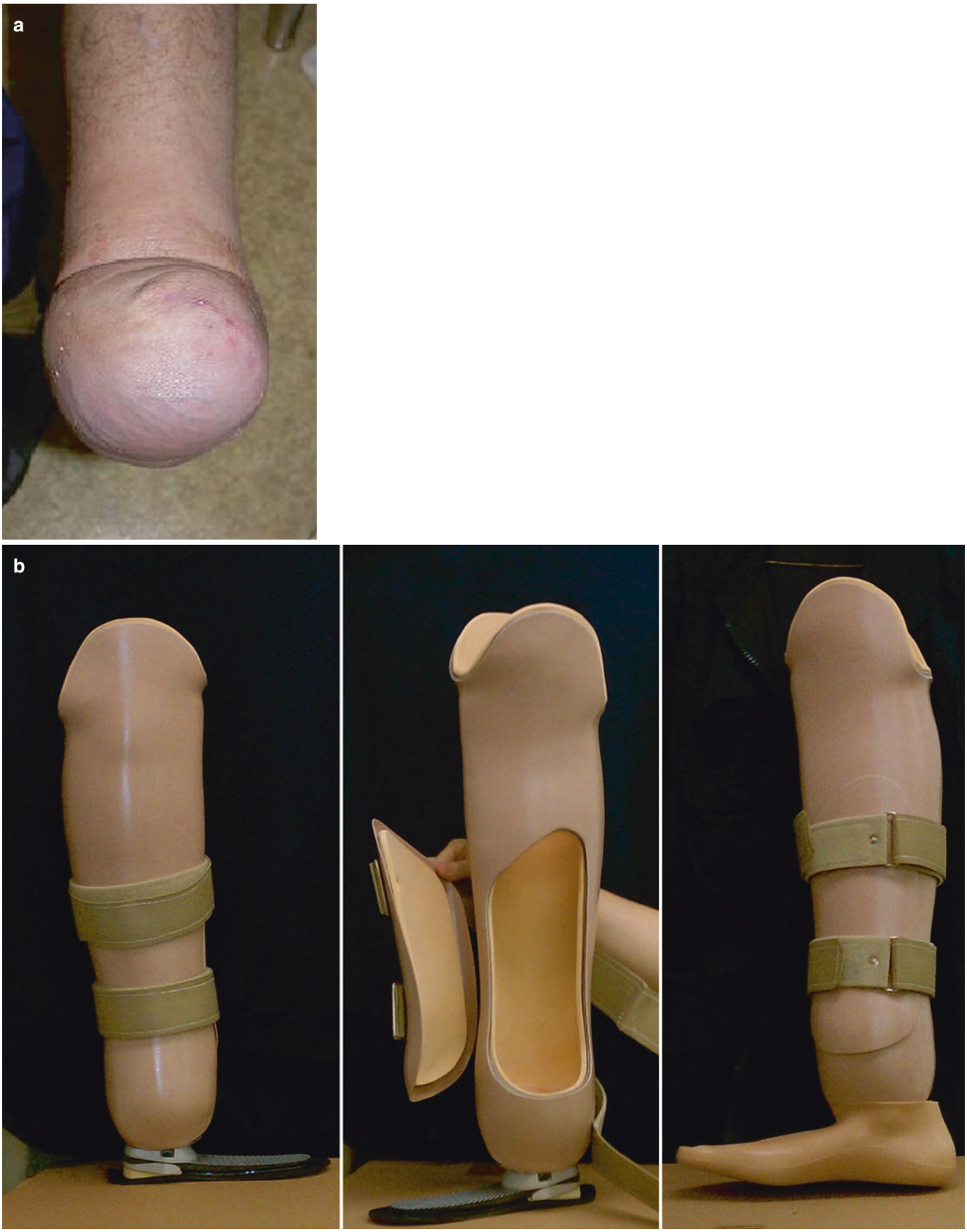

Fig. 23.10 (**a**) A well-performed Syme's amputation with tapered stump and heel pad. (**b**) Syme's prosthesis with and without prosthetic foot

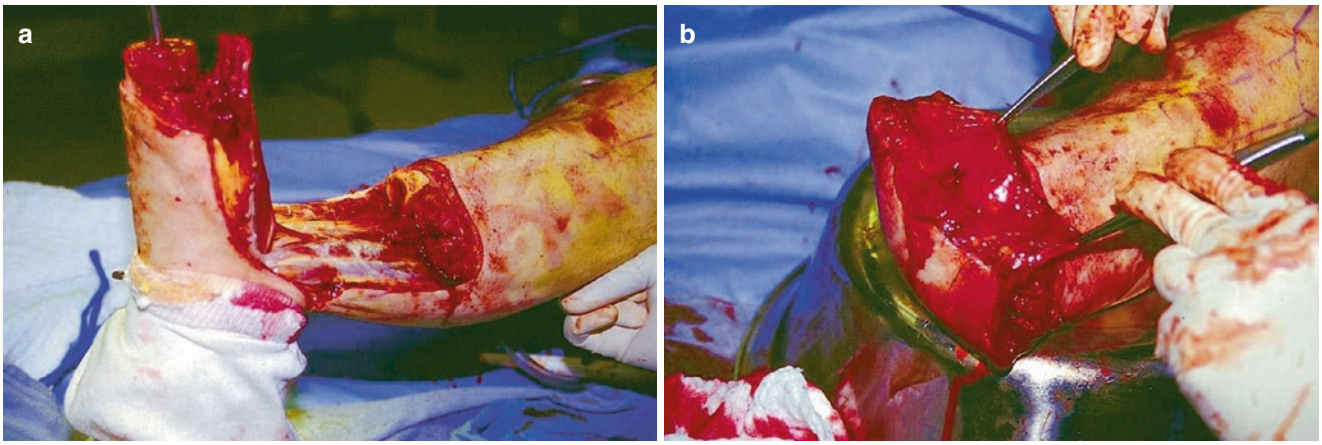

Fig. 23.11 (**a**, **b**) Posterior myofasciocutaneous flap used in transtibial amputation level

Fig. 23.12 Standard below-knee total surface bearing prosthetic socket and silicone suspension sleeve

Postoperative Care

Postoperatively, a rigid plaster dressing is applied [60]. Weight bearing with a prosthesis is initiated at 5–21 days, based on the experience and resources of the rehabilitation team (Fig. 23.12).

Knee Disarticulation

Indications

Knee disarticulation is generally performed in patients with the biologic capacity to heal a surgical wound at the transtibial level, but they are not projected to walk with a prosthesis [61, 62]. In selected patients, it provides an excellent direct load transfer residual limb for weight bearing in a prosthesis. In limited household walkers, or in feeble amputees with limited ambulatory capacity, this level takes advantage of the intrinsically stable polycentric four-bar linkage prosthetic knee joint. The enhanced inherent stability of this prosthetic system decreases the risk for falls in this limited ambulatory population.

Procedure

The currently recommended technique takes advantages of the accepted transtibial posterior myofasciocutaneous flap [63]. The skin incision is made transversely midway between the level of the inferior pole of the patella and the tibial tubercle, at the approximate level of the knee joint.

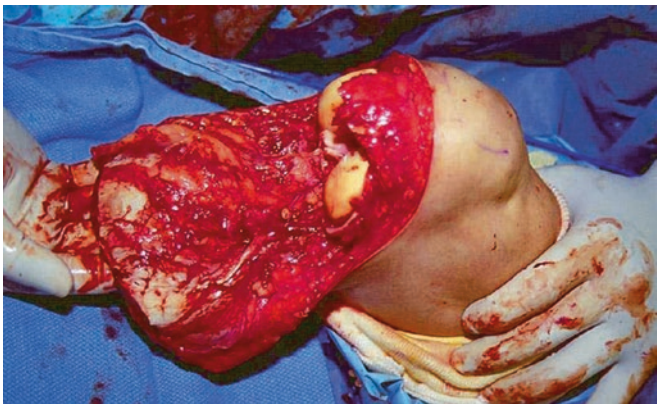

Fig. 23.13 Posterior myofasciocutaneous flap used in knee disarticulation amputation

The length of the posterior flap is equal to a diameter plus 1 cm (as with transtibial). The width of the flap again varies with the size of the patient, ranging between the posterior and middle thirds of the circumference of the leg (Fig. 23.13). The patellar ligament is detached from the tibia, and the capsule of the knee joint is incised circumferentially. The cruciate ligaments are detached from the tibia. A full-thickness posterior myofasciocutaneous flap is created along the posterior surface of the tibia. The soleus muscle is generally removed, unless it is needed to provide bulk. The gastrocnemius muscle is transected at the level of the posterior skin incision, with no creation of a tissue plane between the muscle and skin layers. The patellar ligament is then sutured to the distal stumps of the cruciate ligaments with nonabsorbable suture. The posterior gastrocnemius fascia is then sutured to the patellar ligament and retained knee joint retinaculum. The skin is reapproximated and closed in a layered fashion, and a rigid postoperative dressing/cast is applied.

Postoperative Care

Early weight bearing with a preparatory prosthesis or pylon can be initiated when the tissues of the residual limb appear secure. A locked knee or polycentric four-bar linkage prosthetic knee joint can be used, depending on the walking stability of the patient (Fig. 23.14).

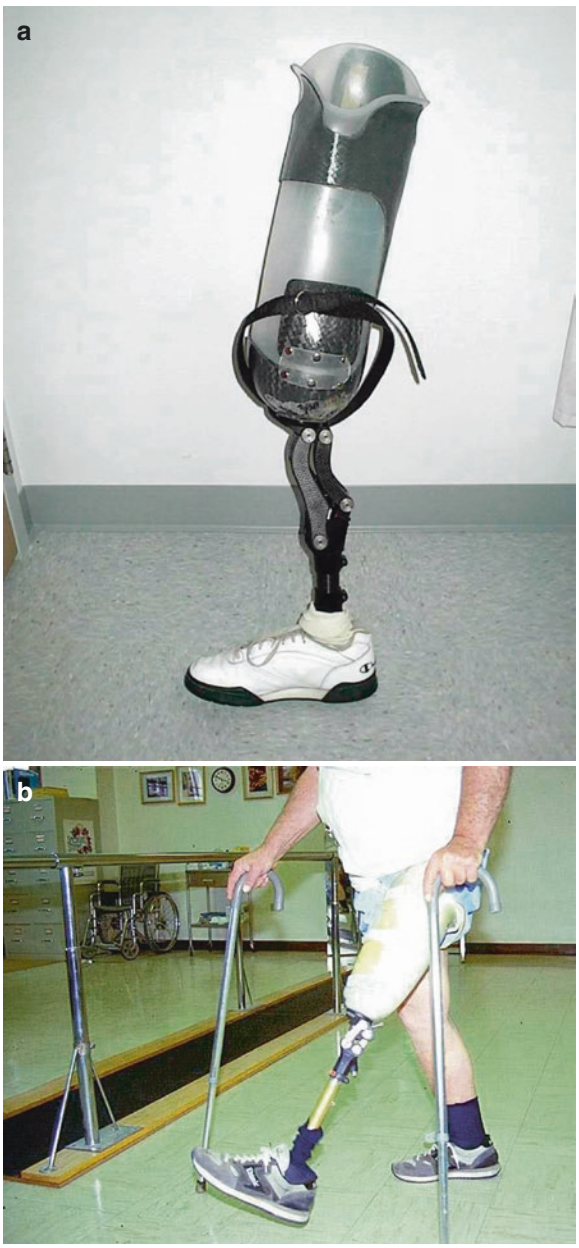

Fig. 23.14 (**a**) Knee diarticulation polycentric four-bar linkage knee joint with preparatory prosthetic. (**b**) Knee disarticulation amputee with polycentric four-bar linkage knee

Transfemoral or Above Knee Amputation

Indications

Gottschalk has clearly shown that the method of surgical construction of the transfemoral residual limb is the determining factor in positioning the femur for optimal load transfer [64]. Standard transfemoral amputation with a fish-mouth incision disengages the action of the adductor musculature. By disengaging the adductor muscles, the femur assumes an abducted, nonfunctional position. This relative functional shortening of the abductors produces an apparently weak abductor gait pattern. By using an adductor-based myocutaneous flap, the adductor muscles can be secured to the residual femur, allowing the femur to be appropriately pre-positioned within the prosthetic socket [65].

Procedure

In order to accommodate a prosthetic knee joint, the optimal bone transection level is 12–15 cm proximal to the knee joint. The soft tissue envelope is composed of a medial-based myofasciocutaneous flap. The flap, including adductor magnus insertion, is dissected off of the femur. After securing hemostasis and cutting the bone, the adductor muscles are secured to the lateral cortex of the femur via drill holes, under normal resting muscle tension. The anterior and posterior muscle flaps are also secured to the residual femur via drill holes. Careful attention is taken to secure the muscles to the residual femur with the hip positioned at neutral flexion-extension, so as to avoid an iatrogenic hip flexion contracture, so often produced by repairing the soft tissues with the residual limb being propped on bolsters during wound closure.

Postoperative Care

An elastic compression dressing is applied, and weight bearing with a preparatory prosthesis is initiated when the wound appears secure (Fig. 23.15).

Hip Disarticulation

Few hip disarticulation amputees become functional prosthetic users. Whether "sitting" in a chair, or "sitting" in a prosthetic socket, the weight-bearing platform can be enhanced by retaining the femoral head within the socket.

Rehabilitation

Surgical amputation should be the first step in the rehabilitation of the patient. Thus, the rehabilitation process should be initiated before the actual amputation surgery, whenever possible. The rehabilitation team should have a reasonable expectation of the patients' ultimate rehabilitation potential. When one measures results from an ambulatory perspective or from a measure of achieving activities of daily living, amputees are less functional or independent with more proximal level amputees. Unilateral ankle disarticulation amputees walk and function at a level very comparable to their age and disease-matched counterparts. While 87% of transtibial amputees will be functional walkers at 2 years, 36% will have died [66]. Ambulatory knee disarticulation amputees fare somewhat less well from both ambulatory and independence perspectives. Very few diabetic, dysvascular transfemoral amputees, or bilateral amputees, will become functional walkers.

Regardless of the amputation level, the first step in regaining functional independence is transfer training leading. Many debilitated patients will not have the energy reserves, stamina, or strength to walk with prosthesis. For these patients, the wheelchair will provide their method of ambulation.

Residual limb care in the early postoperative period can enhance, or detract, from good surgical technique. Specific wound care is related to the circumstances of the surgery. The use of rigid postoperative plaster dressings in transtibial or knee disarticulation amputations controls swelling, decreases postoperative pain, and protects the limb from trauma. The rigid plaster dressing is changed at 5- to 7-day intervals, with early postoperative prosthetic limb fitting and weight bearing being initiated between 5 and 21 days following surgery. Immediate postoperative prosthetic fitting should be reserved

Fig. 23.15 Hybrid transfemoral prosthetic socket with modified quadrilateral shape and ischial containment

for patients with very stable, secure residual limbs. Generally, the residual limb of the transfemoral amputee is managed with a suspended compression dressing. Weight bearing with a prefabricated, or custom, prosthetic socket and training pylon can be initiated when the wound appears secure. With more proximal level amputation, these multiple system-involved individuals are more likely to require walking aids, with almost all dysvascular diabetic amputees requiring the use of a walker or crutches for their limited range of walking.

Following achieving independence in transfer to the chair, the next step is functional ambulation with gait assistive devices. When the patients are allowed to bear weight and start prosthetic fitting will be dependant on the individual patient and the experience of the rehabilitation team. Generally prosthetic fitting for major limb amputation is initiated at 2–6 weeks following surgery.

When the treatment team develops reasonable, realistic goals, patients are capable of achieving the highest level of functional walking compatible with their multiple organ system disease.

Conclusion

Partial foot amputations are frequently used to successfully accomplish limb salvage. If below knee or higher amputation is required to achieve healing, many patients return to community ambulation, still utilizing and stressing the remaining limb. Once any form of amputation has occurred, the patient must be considered at high risk for further amputation [33]. The principles of managing any high-risk foot must be applied, and regular review and management services are essential for preserving the salvaged and contralateral limb.

Patient education, shoe review with appropriate prescription or recommendation, and regular professional foot exams are the mainstay of any preventive program [48]. Regular follow-up must be initiated after healing has been accomplished. The patient should be instructed in regular self foot exams, and the effects of sensory neuropathy. Potentially ulcerative pressure points should be identified and accommodated with orthotics and, or, shoes as needed. Recurring pressure keratosis should be acknowledged as a potential ulceration, and debrided as necessary to prevent the callus from becoming hemorrhagic or ulcerative. This may require intervals as little as every 4 weeks [34, 49].

It has been the authors' experience that no surgical procedure is effective, in itself, in preventing subsequent foot ulcers. The patient with any form of lower extremity amputation must be considered at high risk for further ulceration. Careful clinical follow-up, orthotic/shoe management, and debridement of chronic focal pressure keratosis are far more effective in preventing ulceration or further amputation than any operation.

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Part IV

Organization and Preventive Care

Organization of the Diabetic Foot Care Team

24

John M. Giurini

Abstract

Diabetes is a multifaceted disease characterized by several complications. Patients with lower extremity complications, i.e., peripheral vascular disease and ulcerations, may also suffer with chronic renal disease, cardiac disease, or gastrointestinal disturbances. For this reason, a multidisciplinary team to manage these comorbid complications is essential even when patients are admitted for seemingly unrelated conditions. Failure to recognize and manage these conditions may lead to prolonged hospitalizations and affect outcomes adversely. Clinical practice and reports strongly support the importance and efficacy of the team approach, whether it is for managing foot complications or other complications of diabetes.

Introduction

The prevalence of diabetes continues to be on the rise. In 2012, there were 29.1 million individuals or 9.3% of the American population with diabetes in the United States [1]. Of these, 8.1 million Americans have undiagnosed diabetes. In 2007, the number of Americans with diagnosed and undiagnosed diabetes was 24 million. There are 1.7 million Americans over the age of 20 newly diagnosed with diabetes every year. Under the age of 20, over 18,000 youths are diagnosed with Type 1 diabetes annually and over 5000 are diagnosed with Type 2 diabetes.

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There are also significant racial disparities in the number of individuals with diabetes. Nearly thirteen percent of Hispanic/Latino adults and 13.2% of non-Hispanic black adults are diagnosed with diabetes in the United States. The risk of diagnosed diabetes is 1.2 times greater in Asian Americans, 1.7 times greater in Hispanics, and 1.7 times greater in non-Hispanic blacks than non-Hispanic whites in the United States.

Worldwide the numbers are not much better. In a recent analysis of 200 countries and territories in 21 regions of the world, the total number of adults with diabetes increased from 108 million in 1980 to 422 million in 2014 [2]. Furthermore, the analysis predicts that if the current post-2000 trends continue, the total number of adults with diabetes worldwide will exceed 700 million. The economic burden to these countries is also substantial. Countries with the largest direct cost of care are: China ($170 billion), the USA ($105 billion), India ($73 billion), and Japan ($37 billion). However nearly 60% of the global costs are incurred by low- or middle-income countries where many of these costs are out-of-pocket and lead to severe financial hardships for families. The major driving force for these increases is the obesity and overweight problems worldwide [3–6]. This is a result of the increased consumption and advertising of processed foods, sweetened beverages, and fast foods. The advertising budget of Coca-Cola and Pepsi alone has risen more than tenfold in the past 6 years in the Middle East alone [3]. If the above numbers are not problematic enough, a recent USA Today study of Type 2 diabetes youth between the ages of 10 and 17 showed onset of microvascular and macrovascular complications. The future impact on quality of life, healthcare costs, and productivity can only be surmised.

Because diabetes is a multifaceted disease characterized by a multitude of complications including peripheral vascular disease, ulcerations, chronic renal disease, cardiac disease, or gastrointestinal diseases, it is important that a team of specialists be involved in the care of these patients. It is essential that a multidisciplinary team be available to manage these seemingly unrelated conditions when diabetic patients are admitted to the hospital for foot infections.

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Failure to recognize and manage these comorbid conditions can lead to prolonged hospitalizations and less than optimal outcomes. This is not a new statement or concept [7]. Clinical practice and reports strongly support the importance and efficacy of the team approach, whether it is for managing diabetic foot complications or other complications of diabetes [8–12]. So, the question becomes how does one set up this multidisciplinary team and who should be involved?

Historical Perspective

The modern-day Joslin Diabetes Center was begun in 1952 by Dr. Elliot Joslin (Fig. 24.1). He quickly realized that to successfully treat diabetes and all its potential complications he would need to assemble a team of specialists that understood diabetes and its many complications [13]. For lower extremity problems including infections and peripheral vascular disease, he selected a general surgeon, Dr. Leland McKittrick (Fig. 24.2). For the management of foot problems, he selected Dr. John Kelly who in 1928 became the first podiatrist admitted to the medical staff of a major medical center. This constituted the earliest collaboration between vascular surgery and podiatry, a relationship that continues to this day.

Early collaboration consisted mainly of consultation on challenging cases for radical debridement and amputation in the case of vascular surgery and for conservative management for neuropathic ulcerations in the case of podiatry. However as this collaboration matured, the focus shifted from major limb amputation to limb salvage. Dr. McKittrick and his young new associate, Dr. Frank Wheelock (Fig. 24.3), recognized that not all diabetic patients suffered from peripheral vascular disease. In fact, the majority of below knee amputations were being performed in diabetic patients with peripheral sensory neuropathy and infection. It was this clinical scenario that led to the popularity and feasibility of the transmetatarsal amputation, known as the "Deaconess operation." In his presentation at a surgical meeting in St. Louis, Dr. McKittrick described how it was possible to amputate the forefoot and leave diabetic patients with a stable foot for ambulation [14]. This became a viable alternative to major limb amputation.

Fig. 24.1 Dr. Elliott Joslin, Founder of the Joslin Diabetes Center

Fig. 24.2 Dr. Leland S. McKittrick was chosen by Dr. Elliot Joslin to help with the treatment of the diabetic foot

Limb salvage took another major step forward in the early 80s when infrapopliteal revascularization was introduced. Prior to this time lower extremity bypass procedures were only being performed from the femoral artery to the popliteal artery. However, in diabetic patients, the below knee vessels (dorsalis pedis and posterior tibial) are diseased while the above knee vessels are spared. Therefore the femoral-popliteal bypass did little to improve distal perfusion. The technical ability to perform a femoral or popliteal to dorsalis pedis or posterior tibial bypass using in situ greater saphenous vein meant that many more limbs could be salvaged.

It was also during the early 80s that podiatry became more of a surgical specialty. Even though more limbs were being salvaged through distal bypass techniques, ulcers continued to

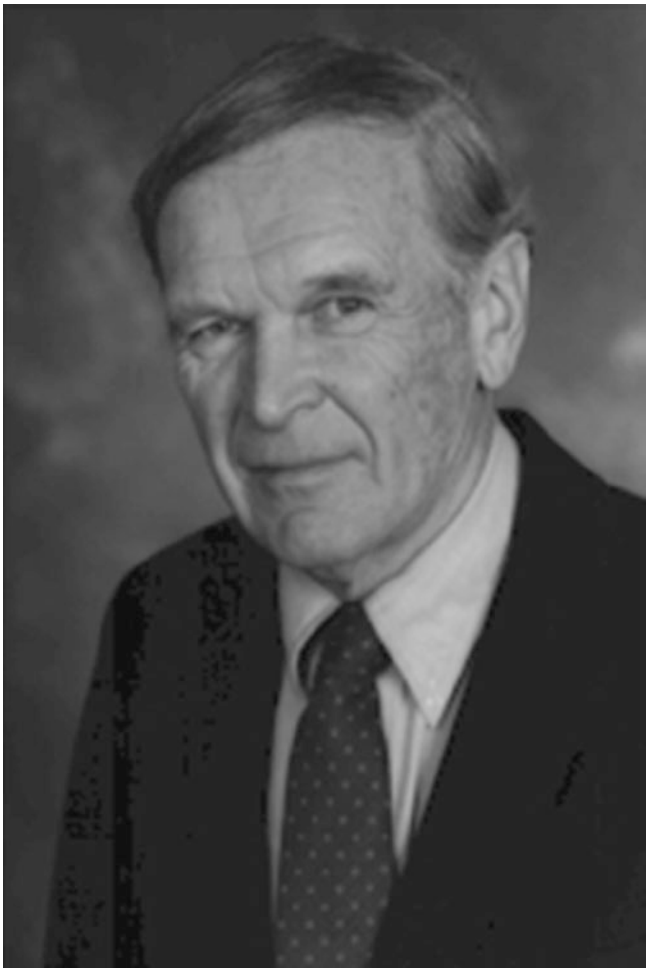

Fig. 24.3 Dr. Frank C. Wheelock, Jr. helped usher in what we know as modern-day vascular surgery

recur in spite of good preventive care. High plantar foot pressures from abnormal foot structure or foot mechanics were responsible for these recurrences. Therefore, the concept of correcting these abnormalities via surgical reconstruction in the form of metatarsal osteotomies, exostectomies, or arthroplasties was discussed. These early procedures were met with much skepticism. However, as patient outcomes were high and recurrence rates were low, foot surgery following bypass surgery became commonplace. So much so that limb-sparing foot surgery following a vascular procedure is now considered the standard of care and not the exception [15, 16].

The Joslin-Deaconess Foot Center Model

One can trace the earliest days of the foot center to the days of Dr. Elliot Joslin when he brought surgeons, podiatrists, and endocrinologists together for the care of diabetic patients. This team approach was formalized nearly 30 years ago when the Joslin-Deaconess Foot Center was formally established. The Center brings together diabetologists, podiatrists, and vascular surgeon to manage diabetic patients with foot ulcers and vascular disease in a more formal coordinated manner.

A question that is commonly asked is "Who should care for the diabetic foot and be involved in the center?" The simple answer is anyone with an interest in the diabetic foot. In reality the answer is more complicated. Care and treatment of the diabetic may go through several phases. There is the care of the acute problem, i.e., acute ischemia, acute infection, or acute Charcot deformity with or without ulceration. There is the chronic problem, i.e., the chronic nonhealing ulceration from ischemia or structural deformity as in chronic Charcot disease with or without ulceration or chronic osteomyelitis. Finally, there is preventive diabetic foot care for those diabetic patients with early complications with or without deformities and who are at increased risk for foot complications. Each of these situations will require a different set of specialists along the course of treatment.

One individual vital to each of these stages is the podiatric physician [17, 18]. This statement is supported by the experience of leading centers around the world. In these centers, the podiatric physician may be the initial contact point for the patient with diabetic foot disease. Often times, it is the podiatric physician who sees the patient most regularly and is the physician who frequently detects the first signs of trouble. The podiatric physician is also most often responsible for the education of the patient in diabetic foot disease and prevention.

The podiatric physician must be well versed in all aspects of diabetic complications and foot care. This should include the evaluation, recognition, and treatment of diabetic foot ulcerations, recognition of peripheral vascular disease, and the recognition and early management of Charcot joint disease. The podiatric physician must also play a significant role in educating the patient and the family on the proper care of the diabetic foot including daily inspection for early signs of tissue breakdown and choice of foot wear [19].

The scope of podiatry varies around the world. In the United States, the podiatric physician provides medical and surgical care of the foot and ankle as defined by the Social Security Act. In other countries podiatric physicians provide only nonsurgical preventive routine care (trimming of nails, corns, and calluses) while in still other countries the profession of podiatry is nonexistent. These are unfortunate circumstances as the podiatric physician in most cases is the most knowledgeable of foot structure and mechanics and therefore able to provide significant insight into surgical approaches to the diabetic foot.

Optimally, the podiatric physician should possess the surgical skills to perform common limb-sparing surgeries such as osteotomies, exostectomies, and metatarsal head resections. Additionally the podiatric physician should possess the knowledge of technically more demanding reconstructive procedures for complex Charcot joint deformities. It is

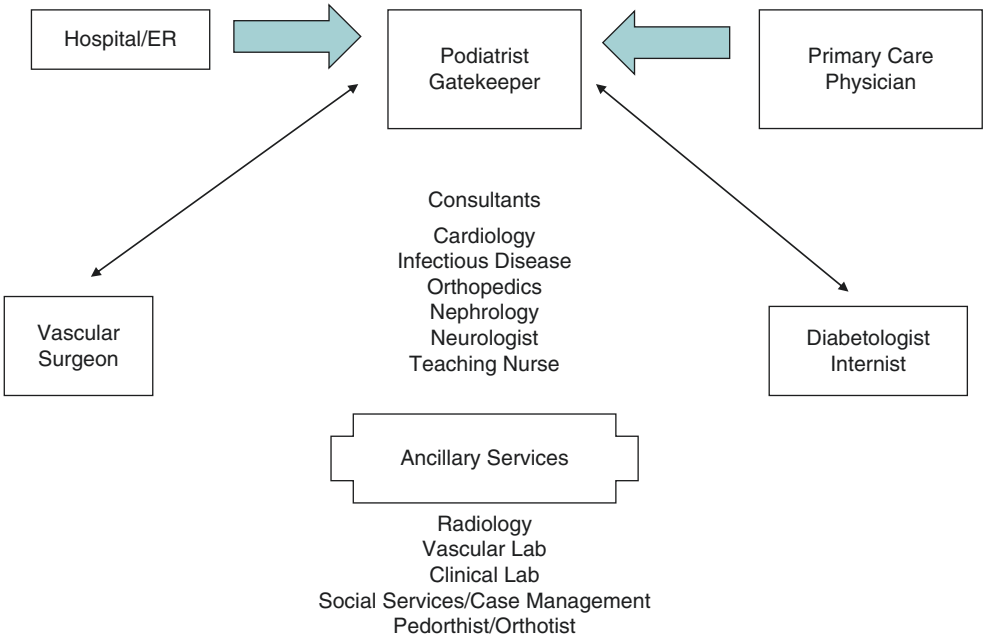

Fig. 24.4 One proposed model for a multidisciplinary diabetic foot clinic utilizes the podiatric physician as the gatekeeper while other specialists are readily available for consultations

not necessary that every podiatric physician possess such skills as long as a member of the multidisciplinary team is identified who possesses these skills.

In addition to the clinical and surgical skills the podiatric physician should possess, they should also be able to educate the patient and their family on the importance of preventive foot care, early recognition of diabetic foot problems and the role complications play and selection of appropriate shoe gear. In the majority of situations, the podiatric physician is responsible for providing long-term management following lower extremity surgery. For this reason, it is recommended that the podiatric physician serves as the gatekeeper of the specialized diabetic foot center (Fig. 24.4). Their ability to evaluate patients regularly and recognize problems early allow them to make timely referrals to the appropriate specialist which is critical.

Just as training and surgical skills of podiatric physicians have changed over the years, so has vascular surgery. In the days of Drs. McKittrick and Wheelock, fellowships in vascular surgery did not exist. Today with the complexity and variety of vascular surgery procedures, fellowships in vascular surgery are essential. Today's vascular surgeons must be trained in both standard open bypass procedures and endovascular procedures which are being performed not only as diagnostic procedures but as definitive therapeutic interventions as well.

In order to provide comprehensive treatment, even in the most complex patients, other key specialists must be readily available for consultation. These include plastic surgery, orthopedic surgery, infectious disease, physical therapy, and prosthetists/orthotists. Each of these specialists is critical to the successful management of diabetic foot disorders.

A plastic surgeon well versed in diabetic foot problems and reconstruction is an invaluable member of the diabetic foot care team. On occasion, the podiatric surgeon and the vascular surgeon will face a wound so large and so deep that healing can take several more months, in spite of successful vascular intervention or reconstructive foot surgery. Therefore, a plastic surgeon knowledgeable, skilled, and willing to perform locally based advancement or rotational flaps can be a tremendous asset to the limb salvage team. His skills should also include the ability to perform free tissue transfers from a remote site to the foot when there is a lack of tissue available locally to make limb salvage feasible.

The Diabetes "Foot" Floor

Success in the management and treatment of diabetic foot disorders is dependent on timely communication by all members of the team. The best way to facilitate this communication is to create a dedicated unit in the hospital for patients with foot problems. This was recognized over 20 years ago by the vascular surgeons and podiatric physicians of the Deaconess Hospital. All patients with lower extremity vascular disease, foot infections, or nonhealing ulcerations are admitted to a common floor. This has several advantages.

First, the vascular surgical team and the podiatry team make morning rounds at the same time every morning. Patients that are being comanaged by the teams are seen together. This assures that communication is timely and direct. It also assures that treatment plans are coordinated between the teams and that both teams are "on the same page" with regard to patient care. This is a fact that is not lost by the patient. The patient quickly recognizes that both teams are working as a unified team for the betterment of the patient.

The second advantage is that consultations can be readily obtained during rounds. Time is not lost waiting for phone calls or emails to be made or returned, orders to be placed, physicians to be notified and see the patient and for recommendations to be made. Physicians are consulted directly while on rounds, patients are seen immediately, diagnostics tests are reviewed or ordered, and recommendations are directly communicated to the referring physicians all in the same morning. This allows for immediate formation and execution of a treatment plan.

The third advantage is rounds can be conducted in a more efficient manner and more patients can be seen. Without a dedicated floor, physicians and residents spend an inordinate amount of time simply traveling from floor to floor, wing to wing, or building to building simply locating patients.

Finally, the fourth advantage of a dedicated unit is the ability to have assigned and appropriately trained nursing staff, case managers, and physical therapists to deal with the special requirements of diabetic patients with wounds. The nursing staff on a dedicated floor is trained to recognize the early signs of infection or graft occlusion. They are trained in appropriate wound care and the performance of dressings changes. They are also familiar with the medical aspects of taking care of diabetic patients such as insulin reactions, managing elevated blood glucose, chest pain, renal disease, special diets, or mobility issues.

Outpatient wound care centers incorporate several of these same characteristics. They are often run in a multidisciplinary fashion by podiatrists, vascular surgeons, general surgeons, or plastic surgeons [20–22]. In some centers, these physicians are present simultaneously while at other centers they may have dedicated times. The centers are also staffed by dedicated wound care nurses who assist the physicians and who serve as the bridge between physicians. They often times will provide direct communication between physicians, staff, and patient to coordinate care. It is imperative that timely consultation and direct communication exist between all personnel regardless of how the center is structured.

The Ultimate Goal

When caring for the diabetic patient with a foot problem, it is important to keep in mind and ask "What is the ultimate goal"? The ultimate goal should always be complete wound healing and limb salvage. Whatever barrier that stands in the way of achieving this goal must be dealt with and overcome. If it is a vascular issue, a vascular surgeon must be consulted and revascularization attempted. If it is an infection issue, an adequate debridement and drainage procedure must be performed. If blood sugars are poorly managed or controlled, endocrinology must be consulted to provide optimal management such that wound healing can be maximized. All of this must be performed in an environment where egos do not get in the way. There is no place for turf battles when it comes to the management of diabetic foot problems.

There must also be a recognized, systematic, and coordinated treatment plan. Patients will quickly pick up when physicians are at odds with each other when it comes to the treatment of their foot. These conflicts do not instill confidence in the patient of their treating physicians. "What should I do?" or "Who do I listen to?" or "Who is right?" are common questions that arise when mixed messages are given. Therefore it is important that if there are disagreements or alternate treatment recommendations that these are resolved before presentation to the patient.

An algorithm is included that represents our joint philosophy on the approach to diabetic foot problems (Fig. 24.5). This algorithm has been developed over the past 25 years from the joint experiences of the vascular surgeons and podiatric physicians of the Joslin-Deaconess Foot Center. Through this algorithm, a systematic approach is provided in the management of the majority of foot problems that we see. Also, because this algorithm was created jointly, there are few disagreements or deviations in the care of these patients. Once again, patients are very astute at recognizing when their treating physicians are in agreement on the care of their foot problem.

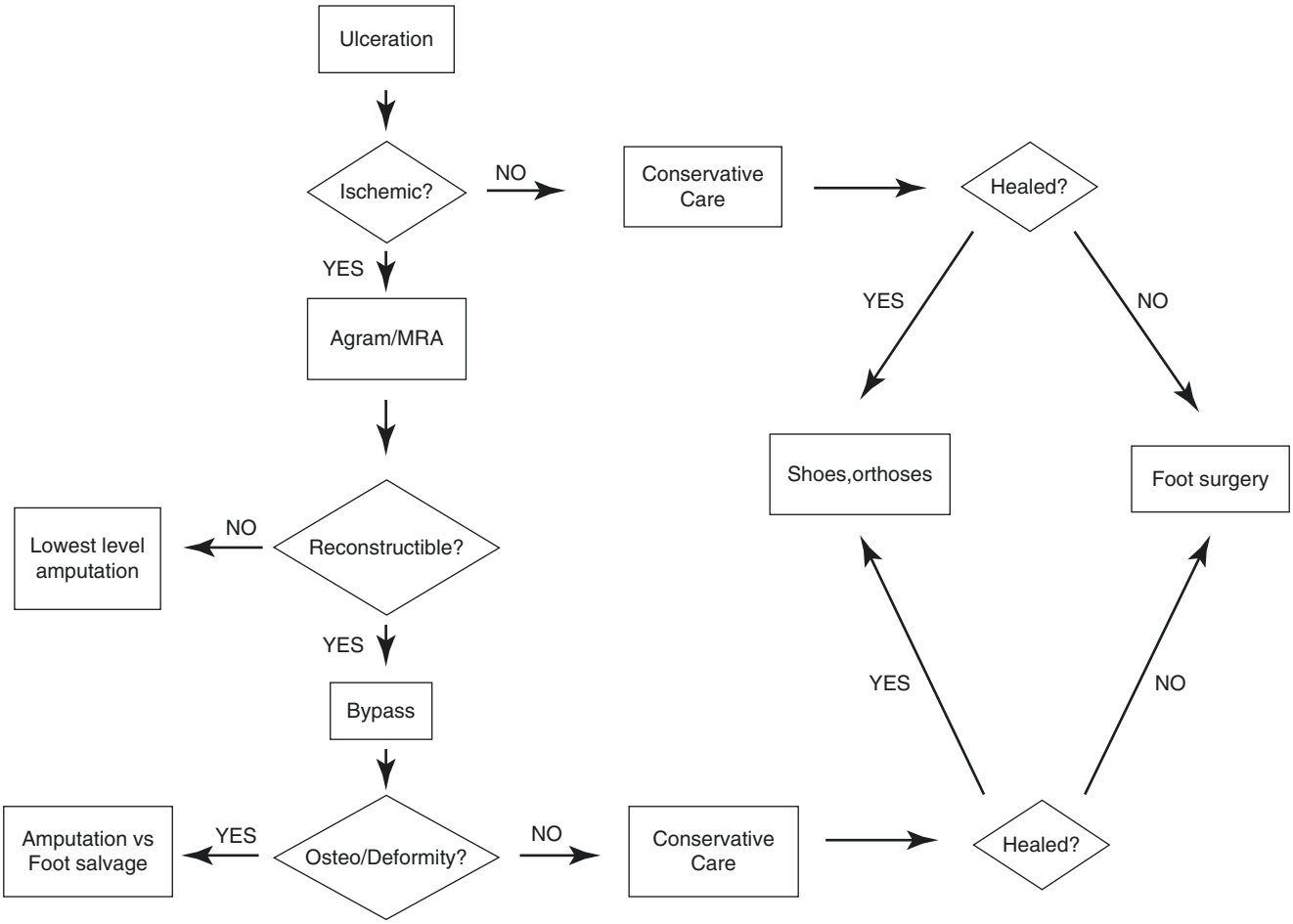

Fig. 24.5 Algorithm from the Joslin-Deaconess Diabetic Foot Center for the management of diabetic foot ulcers©

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Quality of Health Care

25

Marcia A. Testa

Abstract

Delivering the highest quality health care to patients with diabetes requires overcoming complex and multifaceted challenges. However, one area that can contribute to improving health care delivery involves adopting accurate and reliable approaches to performance measurement. The goal of this chapter is to provide the reader with a working knowledge of the conceptual framework of health care quality and performance measurement as applied in the treatment and management of diabetic foot care. A general understanding of the existing systems that monitor diabetes-related quality indicators and outcomes is summarized. Additional information is provided on the promotion of preventive services, reduction of quality gaps, and advancement of scientific knowledge through comparative effectiveness research.

Defining "Quality Health Care"

"Quality health care means doing the right thing at the right time in the right way for the right person and having the best results possible" [1].

This definition of the quality of care developed by the Agency for Healthcare Research and Quality (AHRQ) breaks down the *quality performance construct* into four metrics. Three of the metrics are *process-based measures*, namely, those measures providing the appropriate service at the correct time and performing that service in a satisfactory manner. The fourth measure requires the "best results" *outcomes assessment*. While these measures might seem relatively straightforward, empirical evidence is required to estimate ratings corresponding to appropriateness, timeliness, satisfactory performance, and what constitutes a satisfactory result. Linking the evidence, if it exists at all, to measurement is the key to health care practice performance evaluation. Making the evaluation relevant and useful to practitioners is also a challenging task, especially in specialty areas such as diabetes and podiatry. In a commentary on defining quality health care in podiatry, Wallace questioned current quality measurement practices by thoughtfully asking,

"Maybe this quality paradigm forgets that how one heals, how one listens and the myriad of factors out of our control can impact 'quality'. Does your quality evaluation by the powers that be take those factors into account?" [2].

Although the most common definition of "quality health care" emphasizes that superior health outcomes are the product of superior health care processes, collecting the scientific evidence supporting whether physicians are providing the right treatment at the right time in the right patient is difficult. There are three important terms that should be defined in relation to measuring the quality of care—efficacy, effectiveness, and efficiency [3]. *Efficacy* is the measurement of how well the treatment or intervention works when used under ideal circumstances, namely at the right time, in the right way, and for the right person. Clinical trials which carefully select the right patients for treatment and follow a strict protocol can estimate the efficacy of a treatment. On the other hand, *effectiveness* is the degree of "efficacy" for different levels of the process measures of appropriateness, timeliness, and satisfactory performance ranging from poor to excellent. The assumption is that if the processes are carried out with a rating of excellent, then the health outcome achieved will be equal to the treatment's efficacy. *Efficiency* measures the effect of an intervention in relation to the resources it consumes and is a concept used when evaluating the cost-effectiveness and cost-utility of health care interventions. High quality of care is achieved when the health care processes are delivered ideally such that the highest level of efficacy the treatment can deliver is achieved.

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While most medical procedures and treatments used as the standard of care have been shown to be *efficacious* through rigorous clinical trials, studies demonstrating the differential comparative *effectiveness* among efficacious treatments and interventions that could be used to optimize the quality of health care are relatively few in specialized areas such as diabetes foot care. The goal of this chapter is not to recommend the most effective treatments for diabetes foot care that define high quality, but rather to provide the reader with an introduction to the conceptual framework of health care quality and measurement illustrating with diabetes and foot care examples. Data regarding the associated costs and resources that must be expended to achieve high-quality performance standards is a closely related topic; however, it will not be covered in this chapter. To ensure that all stakeholders communicate effectively about the quality, cost, and value of health care, it is critical that common terminology and nomenclature be used that is understandable and interpretable to a variety of stakeholders including patients, physicians, health plan administrators, politicians, legislators, and government officials. Among these stakeholders, physicians have a particularly critical communications role since they must effectively relate problems and concerns about the current and future state of health care between their patients and most of the other stakeholders.

In 2001, the Institute of Medicine (IOM) published a consensus report, which described six performance goals for high-quality health care, namely that, ".....quality health care is safe, effective, patient-centered, timely, efficient, and equitable" [4]. Since this report, the goals for achieving health quality have not changed appreciably; however, there has been a growing emphasis on directing quality improvement toward more patient-centered outcomes. Patient-centered outcomes are those outcomes most meaningful and important to patients and caregivers [5]. This definition rests on the axiom that patients have unique perspectives that can change and improve the pursuit of clinical questions. The most objective and straightforward health outcomes are survival and length of life. More difficult to ascertain and measure is the quality of the life that is extended through improvements in health care and defining the gradations in the health states and quality of life that are meaningful to patients. There is no question that diabetes shortens the length of life. The number of individuals whose primary cause of death was due to diabetes in 2014 was estimated to be 76,488, making it the seventh leading cause in the United States [6]. In addition, diabetes as a contributing cause is estimated to be over 230,000 deaths per year. Beyond mortality, the growing burden that the complications of diabetes impose on patients is enormous and the associated financial burden on society is not sustainable. A global disease prevention agenda focusing on lifestyle interventions that are safe and effective for preventing diabetes, are associated with improved quality of life, and are cost-effective needs to be the emphasis of future health care quality initiatives [7].

Despite the need for more primary prevention in health programs, the general orientation of the current US health care system is still largely focused on tertiary prevention. Tertiary prevention reduces the impact of current illness and injury by treating individuals to manage chronic health problems to improve their ability to function, their quality of life, and their life expectancy. As such, quality health care initiatives have been relatively limited to reducing medical errors and improving the care and management of individuals who are already far along in their disease. Less emphasis is placed on secondary prevention which aims to reduce the impact of a disease or injury that has already occurred through earlier detection and treatment to halt or slow disease progression and by encouraging lifestyle and behavioral changes to prevent disease or recurrence. To achieve optimal health outcomes, it is apparent that a broader framework should be embraced; one that recognizes that direct medical care is only one determinant of population health outcomes. To prevent, improve, and provide for the ever-expanding diabetes population, future policies must utilize health models that incorporate primary prevention and nonmedical health care determinants. Primary prevention attempts to prevent disease or injury before it ever occurs by preventing exposures to hazards that cause disease or injury, modifying unhealthy behaviors and increasing resilience to disease.

From 1990 to 2009, the rates of diagnosed diabetes per 100 civilian, noninstitutionalized persons in the United States population increased by 217% (from 0.6 to 1.9) for those aged 0–44 years and by 150% (from 5.0 to 12.5) for those aged 45–64 [8]. Rates in these two age groups changed little from 1980 to 1990 and from 2009 to 2014. For those aged 65–74 years, the rates also did not change appreciably from 1980 to 1993; however, they then increased by 113% (from 10.1 to 21.5) from 1993 to 2014. Similarly, for those aged 75 years or older, the rates changed little from 1980 to 1990 and then increased by 140% (from 8.0 to 19.2) from 1990 to 2014. Better detection of undiagnosed persons with diabetes could account for some of this increase; however, the rising rates of obesity have been considered the greatest contributing factor. As shown in Fig. 25.1a, according to The Centers for Disease Control and Prevention's National Health Interview Survey, for January–September 2016, 9.3% (95% confidence interval = 8.83–9.87%) of adults aged 18 and over have been diagnosed with diabetes [9]. This percentage was not significantly different from the 2015 estimate of 9.5%. The prevalence of diagnosed diabetes among adults aged 18 and over increased, from 5.1% in 1997 to 9.2% in 2010, and has since remained stable through 2015. The rates for obesity have paralleled that of diabetes (Fig. 25.1b), except that from 2010 through 2015 have continued to increase [10]. Shifts in demographics in greater

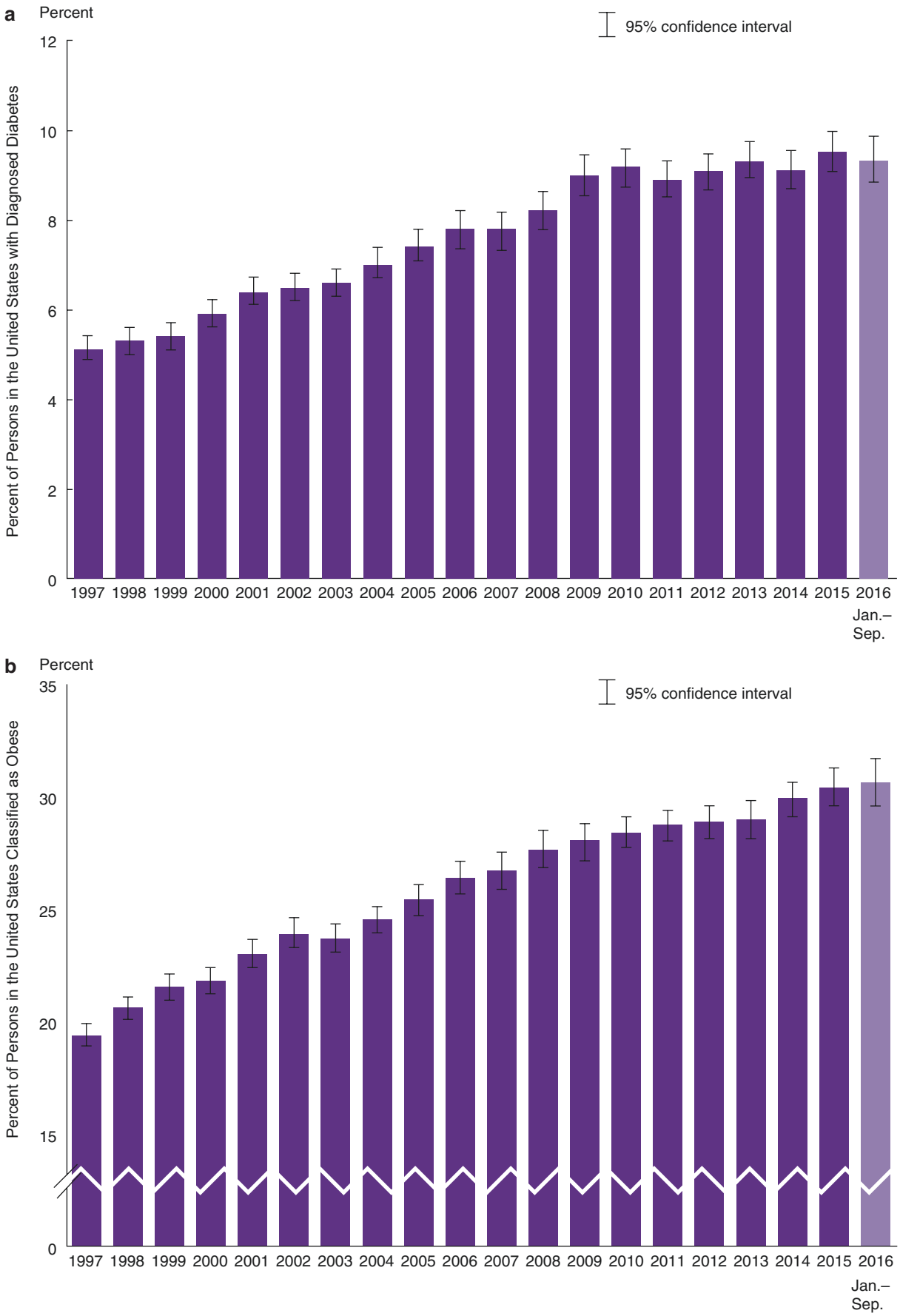

Fig. 25.1 Prevalence of diagnosed diabetes among adults aged 18 and over: United States, 1997–September 2016

numbers of minorities at younger ages might in part be causing the increase in overall obesity rates, but not yet appearing in the diabetes rates since large numbers of younger individuals are undiagnosed. In the most recent 2016 data, non-Hispanic black women (48.1%) were most likely to be obese, compared with Hispanic women (32.2%) and non-Hispanic white women (28.6%). Non-Hispanic black men (34.4%) are more likely to be obese, compared with non-Hispanic white men (30.1%). There was no significant difference in the prevalence of obesity among Hispanic men (31.9%) compared with non-Hispanic black men and non-Hispanic white men. The role of obesity in the increasing prevalence of diabetes is extremely important for diabetes foot care and complications. Obesity and a sedentary lifestyle are risk factors for diabetic foot ulcers and complications [11], and has been shown to be an independent predictor of diabetic foot ulcers (DFUs) [12]. It was found that for every 20 kg unit of higher body weight the odds for a foot ulcer increased by 20% (OR = 1.2, 95% CI = 1.1–1.4). For persons with diabetes within the overweight (BMI 25 to <30), Class 1 Obesity (BMI 30 to <35), Class 2 Obesity (BMI 35 to <40), and Class 3 (>40), there is increasing odds of foot ulcer. Obesity is also a risk factor for poorer foot hygiene and self-care, because of physical restrictions. While substantial research exists on physical activity interventions in adults with diabetes, those at greatest risk for foot ulceration were often excluded or not well represented. Both at-risk patients and their clinicians may be hesitant to increase physical activity because of their perception of diabetic foot ulcer risks.

Primary prevention models propose that the health of a population can be determined through five primary domains: behavioral choices, social circumstances, environmental conditions, genetics, and medical care. To emphasize the relative importance of these domains, consider that it is estimated that 40% of preventable deaths are due to modifiable behaviors such as poor diet, being overweight, low levels of physical activity, and substance abuse [13]. It is apparent that improving access to and the quality of medical care without simultaneously improving the four other health determinants will have a relatively small impact on overall population health. It is also evident that methods used to evaluate health care quality to improve population health must consider the multidimensional nature of health determinants.

How Do We Evaluate Health Care Quality in Diabetes?

Quality improvement initiatives must rely on rigorous evaluation methods. In 1966, Donabedian published a seminal paper proposing effective methods for evaluating the quality of health care [14]. The concepts proposed in his paper have been enduring and are even more relevant today. Donabedian proposed that health care quality could be assessed through three primary measurement domains, namely, structure, process, and outcomes. Structure measurements focus on the environment and support where health care services are provided, such as hospital buildings, equipment, and staff. As mentioned above, process measures include the actual steps needed to carry out the health care services of interest such as physical examinations, laboratory testing, surgery, and pharmacologic therapies. Outcome measures focus on the end results of the health care services delivered including laboratory results, levels of physical and cognitive functioning, quality of life, morbidity, and mortality. Each of these quality measures has positive and negative attributes related to our ability to measure and utilize them for quality improvement, but if applied concurrently, an even more accurate evaluation can be made. Table 25.1 gives some examples of measures of structure, process, and outcome relevant to surgery of the foot and ankle.

One's point of reference and perspective for evaluation can heavily influence perceptions of achievement and failure in health care quality. This is also true for specific disease management programs including diabetes-related care. Stakeholders representing a wide range of values often view quality of care from different perspectives. For example, while an endocrinologist might identify patient adherence to a diabetes medication regimen as an important indicator of health care quality, an insurer might place a greater emphasis on the length of a hospital stay for uncontrolled diabetes. Furthermore, the patient might expect that better health care should improve their ability to function in their daily lives, while a public health official might focus on whether educational campaigns are able to prevent hospitalizations. These varying perspectives make the evaluation of health care quality difficult to standardize across diverse groups of stakeholders.

Historically, the surgeon's perspective of health care quality has been heavily influenced by three individuals, Donabedian, Codman, and Khuri [15]. Ernest Amory Codman, M.D., was one of the most important figures in the history of outcomes research in medicine. His work foreshadowed many of today's most pressing issues in assessing the quality of care. Codman contributed to the field by creating the concept of "end results" where hospitals analyze treatment *outcomes* to improve health care quality [16]. Using this end results approach, all surgical cases are reviewed and complications assigned a root cause of either patient and/or surgical selection, surgeon technique and/or instrumentation, pre- or postoperative patient management, or patient comorbidities. Codman's innovation in quality monitoring contributed to his appointment as Chair of the Committee for the Standardization of Hospitals, now known as the Joint Commission, and also known as the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). The Joint Commission on Accreditation of

Table 25.1 Structure, process, and outcome measures of health care quality

Quality measure	Examples	Advantages	Disadvantages
Structure	– Hospital, clinic facilities – Proper equipment, operating room facilities and efficiency – Proper training of physicians, nurses, etc. – Adequate administrative staff	– Usually easy to gather data – Data is often objective (i.e., billing codes, administrative information)	– May be difficult to determine the extent of the relationship between the structure measurement and process and outcome measurements
Process	– Complete and timely screening examinations, exam skills – Proper referrals and use of multidisciplinary teams – Surgical technique – Proper choice of diagnostic tests and treatment choices	– May answer more relevant questions – Provides timely results for decision making	– May not relate to outcomes (improved process may not result in improved outcome)
Outcome	– Survival – Amputation – Success rates of surgical procedures	– Often easier to interpret – Often considered a more valid measurement – Often objective measurements are available	– Choice of outcome may be immaterial (treatment may decrease amputation rates but may also decrease functionality and patient quality of life) – May not be feasible or possible to determine causes of long-term outcomes such as amputations – May not be possible to compare or produce timely results when decisions must be made – Certain outcomes are less objective or not applicable to certain patient populations (i.e., patient satisfaction, classifying outcomes as good or poor, instruments such as American Orthopedic Foot and Ankle Society (AOFAS) scores)

Healthcare Organizations is a private, not-for-profit organization established in 1951 to evaluate health care organizations that voluntarily seek accreditation. The Joint Commission evaluates and accredits more than 16,000 health care organizations in the United States, including 4400 hospitals, more than 3900 home care entities, and over 7000 other health care organizations that provide behavioral health care, laboratory, ambulatory care, and long-term care services [17]. The Joint Commission also evaluates and accredits health plans and health care networks. It is governed by representatives from the American College of Physicians, the American College of Surgeons, the American Dental Association, the American Hospital Association, the American Medical Association, an at-large nursing representative, six public members, and the Joint Commission President. JCAHO was originally established to standardize minimum quality health care provided by US hospitals. In 1996, JCAHO began recognizing health care organizations that utilize process and outcome measures for quality improvement, through the development of the Codman Award [15, 18]. One of the Joint Commission's programs, Pioneers in Quality (PIQ) assist hospitals seeking to adopt electronic clinical quality measures [19]. The program includes educational programs (e.g., webinars for CEUs), a resource portal, recognition categories, an advisory council, a modified annual report, speaker's bureau outreach, a peer-to-peer solution exchange, as well as having a strong focus on partnering with hospitals to provide the highest level of quality care for patients and their families.

Khuri is known for leading the initial development of a Department of Veterans Affairs prospective surgical surveillance system. In the mid-1980s the Veterans Health Administration (VHA) was criticized for their high operative mortality. To that end, Congress passed Public Law 99–166 in December 1985 which mandated that the VHA report their surgical outcomes in comparison to national averages and that their data be risk-adjusted to account for the severity of illness of the VHA surgical patient population. In 1991, the National Veterans Affairs Surgical Risk Study (NVASRS) began in 44 Veteran's Administration Medical Centers. After several modifications and name changes, this system eventually became the National Surgical Quality Improvement Program (NSQIP), which is still used today to monitor health care outcomes of surgical morbidity and mortality throughout the United States [20].

Quality of Care in Diabetic Limb Management

To understand how the quality of diabetes health care is measured, a general understanding of existing systems that monitor diabetes-related quality indicators and outcomes is necessary. These systems involve federal, state, and regional governmental agencies, health insurance companies, and specialized professional organizations and institutions. At the federal level, the Department of Health and Human Services (HHS) in the United States, which operates under

the executive branch of government, is the primary agency charged with protecting health. All health-related programs and offices are coordinated through the Office of the Secretary of HHS and its agencies [21]. As of April 2017, HHS had 11 operating divisions, including eight agencies in the U.S. Public Health Service and three human services agencies. These entities administer a wide variety of health and human services and research programs. Six of the most well-known agencies that play important roles in monitoring health care and improving health are listed below:

- Agency for Healthcare Research and Quality (AHRQ) studies utilization and cost of health care services, develops and studies quality measures, disseminates health outcomes research to improve health care quality, and supports evidence-based medicine.
- Centers for Disease Control and Prevention (CDC) prevents and controls disease both nationally, through departments of public health, and internationally; surveys patient safety and health care quality.
- Centers for Medicare and Medicaid Services (CMS) provides health care coverage to older, disabled, and low-income Americans comprising 25% of the US population.
- Food and Drug Administration (FDA) is responsible for ensuring the safety and effectiveness of vaccines, medications and other biologic products, products that emit radiation, medical devices, and food.
- Health Resources and Services Administration (HRSA) is part of the Public Health Service and provides health care to people who are geographically isolated, economically or medically vulnerable.
- National Institutes of Health (NIH) is the primary federal research agency and has the largest source of medical research funding worldwide.

Each of these entities utilizes data-driven approaches to monitor health care quality through specialized programs and projects. For example, the AHRQ's Healthcare Cost and Utilization Project (HCUP) provides states and organizations public access databases and software tools to enhance their health care quality improvement projects [22]. The HCUP databases bring together the data collection efforts of State data organizations, hospital associations, private data organizations, and the Federal government to create a national information resource of patient-level health care data. HCUP includes the largest collection of longitudinal hospital care data in the United States, with all-payer, encounter-level information beginning in 1988. HCUP databases include:

- The National (Nationwide) Inpatient Sample (NIS) containing data on more than seven million hospital stays each year. Weighted, it estimates more than 35 million hospitalizations nationally.
- The Kids' Inpatient Database (KID) is the only all-payer pediatric inpatient care database in the United States, containing data from two to three million hospital stays.
- The Nationwide Emergency Department Sample (NEDS) is the largest all-payer emergency department (ED) database in the United States, yielding national estimates of hospital-based ED visits. Unweighted, it contains data from approximately 30 million ED visits each year. Weighted, it estimates roughly 135 million ED visits.
- The Nationwide Readmissions Database (NRD) is a unique and powerful database designed to support various types of analyses of national readmission rates for all payers and the uninsured. Unweighted, the NRD contains data from approximately 15 million discharges each year. Weighted, it estimates roughly 35 million discharges.
- The State Inpatient Databases (SID) contain the universe of inpatient discharge abstracts from participating states and encompasses about 97% of all US community hospital discharges
- The State Ambulatory Surgery and Services Databases (SASD) include data for ambulatory surgery and other outpatient services from hospital-owned facilities. The databases contain a core set of clinical and nonclinical information on all patients, regardless of payer, including those covered by Medicare, Medicaid, private insurance, and the uninsured.
- The State Emergency Department Databases (SEDD) contain data from hospital-affiliated emergency departments for visits that do not result in hospitalizations.

In addition, the HCUP Quality Improvement (QI) metrics measure multiple health care dimensions that allow policy makers, stakeholders, researchers, and physicians to identify quality gaps and track health care quality over time. The QIs are divided into four modules: Prevention Quality Indicators, Inpatient Quality Indicators, Patient Safety Indicators, and Pediatric Quality Indicators. Software and user guides for all four modules are available to assist users in applying the Quality Indicators to their own data. These QIs analyze available inpatient hospital discharge data and they also extrapolate those results to identify ways to improve quality of preventive and outpatient care for a variety of health care conditions including "ambulatory care sensitive conditions" or ACSCs. There is a total of 14 ACSCs, and four ACSCs related specifically to diabetes, namely: (1) diabetes short-term complication admission rate; (2) diabetes long-term complication admission rate; (3) uncontrolled diabetes admission rate; and (4) rate of lower-extremity amputation among patients with diabetes [23]. In addition to being able to download data and software, easy-to-use online tools are also available. For example, if an individual were interested

Table 25.2 Hospital inpatient national statistics obtained using HCUPnet online query tool

Year	Number of discharges	Percent of discharges	Discharges per 100,000 persons	Age (mean)	Length of stay, days (mean)	% Died	% Male	Charges,[a] $ (mean)	Costs, $[a] (mean)	National Aggregate charges, (billions $)[a]	National Aggregate costs, (billions $)[a]
2004	111,110	29.6%	37.9	65.4	11.2	3.8	60.9	59,133	21,841	6.57	2.42
2014	119,245	33.7%	37.4	62.5	9.6	1.8	69.0	80,960	20,841	9.66	2.49

[a]2014 Dollars

in statistics on lower limb amputations nationally, or for each state individually, these statistics could be easily accessed without any database downloading or statistical packages through the HCUPnet online application [24]. To illustrate, using the HCUPnet "Get Quick Statistics Table" function, a simple query was run (selecting options: "inpatient," "national," year = 2004, then = 2014, "specific diagnosis or procedure = no") to obtain information on the number of discharges and other statistics from inpatient hospitals stays due to all procedures including lower limb amputations for the years 2004 and 2014. The results for lower limb amputations are displayed in Table 25.2. These statistics are weighted national estimates from HCUP National (Nationwide) Inpatient Sample (NIS) [2004 and 2014], based on data collected by individual States and provided to AHRQ by the States. Out of 231 inpatient procedures, lower limb amputations ranked the 47th and 45th most common procedure in 2004 and 2014, respectively. While the costs decreased by 4.6% over the 10-year period from 2004 to 2014, the charge-to-cost ratio increased from 2.7 to 3.9. The mean age decreased by 3 years and the proportion of males increased by 8.1%. The percent dying decreased by more than 50%, from 3.8 to 1.8%, while the length of stay decreased by 14%, from 11.2 to 9.6 days. If more detailed analyses are required, this is also possible. For example, one could compare the length of stay and charge for Massachusetts versus another state or the national average. For Massachusetts versus nationally, in 2013 (latest year available), the mean age was 65.0 versus 62.3 years, length of stay 8.8 versus 9.4 days, charges $51,981 versus $76,415, costs $21,992 versus $20,427, and percent died 1.76 versus 1.78.

Another AHRQ data source for monitoring quality is the *Medical Expenditure Panel Survey (MEPS)* [25] which includes large-scale surveys of families and individuals, their medical providers, and employers across the United States. MEPS is the most complete source of data on the cost and use of health care and health insurance coverage. Using the 2014 MEPS interactive data table generator, it was found that 63.4% of persons with diabetes reported having an HbA1c measurement taken within the past year, compared to 65% in 2004. In 2014, Hispanics and Blacks had HbA1c measurement rates nearly 20% lower than Whites, while 10 years earlier the rates were approximately 15% lower (Table 25.3). Lower income and poorer health also appeared to be predictive of a lower probability of having HbA1c measures taken in both 2014 and 2004. In 2014, a total of 89.8% of persons with diabetes reported having a cholesterol check, and poorer health status was positively correlated with having a cholesterol measurement taken, while poverty did not differentiate. A total of 68.1% of persons with diabetes reported having a retinal exam. As shown in Table 25.4, in 2014, 73.4% of patients with diabetes reported having a foot exam, up from 60% in 2004. Racial and ethnic and economic disparities were not as high for diabetes foot exams compared to disparities for HbA1c measurement. The 13.4% improvement in the frequency of foot exams as compared to the lack of improvement for the frequency of HbA1c measurement over the 10-year period between 2004 and 2014 has not been investigated, but may be due to some degree to a greater focus by clinicians on the importance of foot exams for signaling worsening of disease and the potentially devastating effects of lower limb amputations on quality of life. Similar to HCUPnet, the MEPS database can be analyzed using the MEPSnet Query Tools that allow detailed table generation across all variables in the survey.

Since 2003, the AHRQ has produced annually the National Healthcare Quality Report and the National Healthcare Disparities Report. Starting in 2014, findings on health care quality and health care disparities were integrated into a single report, the National Healthcare Quality and Disparities Report (NHQDR) [26]. The NHQDR provides an overview of the quality of health care received by the general US population and disparities in care experienced by different racial, ethnic, and socioeconomic groups. The report is based on more than 250 measures of quality and disparities covering a broad array of health care services and settings. The NHQDR uses both process and outcome measures to assess and evaluate the quality of diabetes care in the USA. Diabetes care process measures include whether an HbA1c test, retinal eye exam, influenza immunization, and foot examination were performed in the last year, while outcome measures include actual test results (e.g., HbA1c > 9.5% is poor, <9% needs improvement, <7% good; total cholesterol <200 mg/dL is good; percent with blood pressure < 140/90 mm/Hg) and classification of "avoidable" hospitalizations. Here hospitalization could be considered an indicator of the health outcome "worsening physical health status." Avoidable hospitalizations have been defined as persons with diabetes

Table 25.3 Percent of adults age 18 and over with diabetes who reported having a hemoglobin A1C measurement at least once in past year: United States, 2014

Population characteristic	Population total (in thousands)	Had measurement		Did not have measurement		Don't know		Non-response	
		%	S.E.	%	S.E.	%	S.E.	%	S.E.
Total	24,589	63.4	1.3	7.3	0.7	12.2	0.9	17.1	1.1
Age in years									
18–64	13,345	65.6	1.8	8.3	0.9	11.0	1.2	15.2	1.3
18–44	2762	65.7	3.5	7.2	2.0	11.8	2.3	15.3	2.8
45–64	10,584	65.5	2.0	8.6	1.1	10.8	1.4	15.1	1.5
65 and over	11,243	60.8	2.3	6.1	1.1	13.7	1.4	19.4	1.7
Sex									
Male	11,726	65.1	1.8	6.7	1.0	12.1	1.4	16.0	1.5
Female	12,863	61.8	1.9	7.8	1.1	12.3	1.3	18.1	1.5
Race/ethnicity									
Hispanic	3813	50.5	3.1	11.0	1.7	12.3	1.7	26.2	2.5
White, Non-Hispanic	14,979	69.5	1.9	5.1	0.9	10.8	1.3	14.6	1.5
Black, Non-Hispanic	3626	52.3	2.1	9.1	1.5	16.3	1.7	22.3	1.8
Amer. Indian/AK Native/Multi. Races, non-Hispanic	--	--	--	--	--	--	--	--	--
Asian/Hawaiian/Pacific Islander, non-Hispanic	1508	63.4	4.7	13.7	3.8	15.5	3.3	7.3	2.2
Health insurance status[a]									
<65, Any private	8320	69.4	2.4	6.9	1.1	10.3	1.8	13.4	1.6
<65, Public only	3582	61.6	3.4	7.0	1.5	13.4	2.2	17.9	2.2
<65, Uninsured	1443	53.3	5.4	19.4	4.5	8.9	2.6	18.5	2.8
65+, Medicare only	3798	50.4	3.6	7.9	2.1	20.0	3.1	21.7	2.8
65+, Medicare and private	5376	71.8	3.1	4.4[c]	1.5[c]	8.0	1.4	15.8	2.2
65+, Medicare and other public	1996	51.9	4.5	7.6	2.1	16.8	2.9	23.8	3.7
65+, No Medicare	--	--	--	--	--	--	--	--	--
Poverty status[b]									
Negative or poor	3961	53.8	3.2	10.3	1.9	14.1	2.1	21.8	2.2
Near-poor	1412	47.3	4.6	8.4[c]	2.8[c]	19.2	3.7	25.1	5.1
Low income	4185	56.6	3.2	7.8	1.5	18.6	2.7	17.0	2.2
Middle income	6813	66.0	2.3	7.0	1.4	10.7	1.7	16.3	1.8
High income	8218	72.0	2.6	5.7	1.3	8.2	1.6	14.1	1.9
Census region									
Northeast	4014	61.8	3.5	7.6	2.0	15.6	3.1	15.0	2.2
Midwest	5535	70.6	3.7	4.3[c]	1.6[c]	9.1	2.0	16.0	2.4
South	9985	61.3	1.6	7.3	1.0	12.9	1.4	18.5	1.8
West	5055	60.8	2.9	10.4	1.6	11.7	1.9	17.0	2.2
Perceived health status									
Excellent	1318	63.8	6.0	9.6[c]	2.9[c]	11.5[c]	3.6[c]	15.1	3.7
Very good	5204	68.0	2.9	7.3	1.7	10.1	1.8	14.6	2.2
Good	9110	64.4	2.3	7.5	1.3	10.8	1.6	17.3	1.8
Fair	6696	60.3	2.3	6.3	0.9	13.9	1.8	19.5	1.8
Poor	2260	57.3	4.3	8.2	2.4	18.8	3.1	15.7	3.2
Footnotes:									

[a]Uninsured refers to persons uninsured during the entire year. Public and private health insurance categories refer to individuals with public or private insurance at any time during the period; individuals with both public and private insurance and those with Tricare (Armed-Forces-related coverage) are classified as having private insurance

[b]Poor refers to incomes below the Federal poverty line; near poor, over the poverty line through 125% of the poverty line; low income, over 125% through 200% of the poverty line; middle income, over 200–400% of the poverty line; and high income, over 400% of the poverty line

[c]Relative standard error equal to or greater than 30%

—Less than 100 sample cases

Source: Center for Financing, Access and Cost Trends, Agency for Healthcare Research and Quality: Medical Expenditure Panel Survey, 2014

Internet Citation:

Agency for Healthcare Research and Quality. Table 1.1: Percent of Adults Age 18 and Over with Diabetes who Reported Having a Hemoglobin A1C Measurement at Least Once in Past Year: United States, 2014. Medical Expenditure Panel Survey Household Component Data. Generated interactively. (April 26, 2017)

Table 25.4 Percent of adults age 18 and over with diabetes who reported having a foot examination in past year: United States, 2014

Population characteristic	Population total (in thousands)	In the past year		More than 1 year ago		Never had foot checked		Non-response	
		Percent	Standard error	Percent	Standard error	Percent	Standard error	Percent	Standard error
Total	24,589	73.4	1.2	7.2	0.7	17.5	1.1	1.9	0.4
Age in years									
18–64	13,345	69.6	1.6	7.4	1.0	21.2	1.5	1.8[c]	0.7[c]
18–44	2762	60.8	4.0	7.9[c]	2.4[c]	30.9	3.8	0.4[c]	0.3[c]
45–64	10,584	71.8	1.8	7.3	0.9	18.7	1.6	2.2[c]	0.9[c]
65 and over	11,243	78.0	1.7	6.9	1.1	13.1	1.3	2.0	0.5
Sex									
Male	11,726	74.6	1.7	6.6	1.0	16.8	1.4	2.1	0.6
Female	12,863	72.4	1.8	7.7	1.0	18.2	1.6	1.7	0.5
Race/ethnicity									
Hispanic	3813	66.6	2.8	7.0	1.3	23.1	2.3	3.4	0.9
White, Non-Hispanic	14,979	76.2	1.7	6.4	0.9	15.5	1.5	1.9[c]	0.7[c]
Black, Non-Hispanic	3626	73.4	2.3	9.5	1.7	16.1	2.0	1.0[c]	0.4[c]
Amer. Indian/AK Native/ Multi. Races, non-Hisp.	--	--	--	--	--	--	--	--	--
Asian/Hawaiian/Pacific Islander, non-Hispanic	1508	64.7	5.4	9.8[c]	3.3[c]	25.4	5.4	0.0[c]	0.0[c]
Health insurance status[a]									
<65, Any private	8320	70.8	2.2	6.9	1.4	20.3	2.0	1.9[c]	1.1[c]
<65, Public only	3582	69.1	2.9	8.4	1.6	20.8	2.5	1.6[c]	0.8[c]
<65, Uninsured	1443	63.5	5.0	8.0[c]	2.7[c]	27.1	4.3	1.4[c]	0.7[c]
65+, Medicare only	3798	78.1	2.8	6.8	1.9	12.9	2.2	2.2[c]	0.9[c]
65+, Medicare and private	5376	79.3	2.6	7.8	1.7	10.9	2.1	2.1[c]	0.8[c]
65+, Medicare and other public	1996	73.9	3.5	5.0[c]	1.6[c]	19.7	3.1	1.5[c]	1.0[c]
65+, No Medicare	--	--	--	--	--	--	--	--	--
Poverty status[b]									
Negative or poor	3961	71.7	2.4	6.5	1.3	19.4	2.2	2.3[c]	0.8[c]
Near-poor	1412	78.1	4.0	6.4[c]	2.7[c]	14.2	3.2	1.3[c]	1.1[c]
Low income	4185	68.8	2.6	9.3	1.8	19.7	2.1	2.2[c]	0.9[c]
Middle income	6813	74.7	2.2	7.2	1.6	16.7	1.8	1.5[c]	0.5[c]
High income	8218	74.8	2.5	6.6	1.2	16.7	2.1	1.9[c]	1.1[c]
Census region									
Northeast	4014	77.5	2.8	6.2	1.6	15.3	2.2	1.0[c]	0.7[c]
Midwest	5535	75.5	2.7	5.1	1.4	16.6	2.3	2.8[c]	1.7[c]
South	9985	72.3	1.8	8.1	1.1	18.1	1.5	1.5	0.4
West	5055	70.2	2.6	8.4	1.5	19.1	2.6	2.3	0.5
Perceived health status									
Excellent	1318	72.0	5.8	3.5[c]	1.5[c]	24.3	5.8	0.2[c]	0.2[c]
Very good	5204	67.1	3.2	7.4	1.5	22.3	2.5	3.1[c]	1.8[c]
Good	9110	72.8	1.7	8.8	1.4	16.3	1.7	2.1	0.5
Fair	6696	77.9	2.2	5.8	1.2	15.2	1.7	1.1[c]	0.5[c]
Poor	2260	77.8	3.1	6.2	1.8	14.4	2.8	1.5[c]	1.0[c]

[a]Uninsured refers to persons uninsured during the entire year. Public and private health insurance categories refer to individuals with public or private insurance at any time during the period; individuals with both public and private insurance and those with Tricare (Armed-Forces-related coverage) are classified as having private insurance

[b]Poor refers to incomes below the Federal poverty line; near poor, over the poverty line through 125% of the poverty line; low income, over 125% through 200% of the poverty line; middle income, over 200–400% of the poverty line; and high income, over 400% of the poverty line

[c]Relative standard error equal to or greater than 30%

—Less than 100 sample cases

Note: Estimates were generated using the DCS weight variable

Source: Center for Financing, Access and Cost Trends, Agency for Healthcare Research and Quality: Medical Expenditure Panel Survey, 2014

Internet Citation:

Agency for Healthcare Research and Quality. Table 1.4: Percent of Adults Age 18 and Over with Diabetes who Reported Having a Foot Examination in Past Year: United States, 2014. Medical Expenditure Panel Survey Household Component Data. Generated interactively. (April 26, 2017)

admitted with uncontrolled diabetes without complications (absence of short- or long-term complications or lower extremity amputation). Short-term complications are defined to include ketoacidosis, hyperosmolarity, and coma. Long-term complications include renal, eye, neurologic, circulatory, or other unspecified diagnosis related to diabetes.

Since 2011, a new survey conducted by the Centers for Disease Control and Prevention, the National Hospital Care Survey (NHCS), integrates inpatient data formerly collected by the National Hospital Discharge Survey (NHDS) with the emergency department, outpatient department, and ambulatory surgery center data collected by the CDC's National Hospital Ambulatory Medical Care Survey (NHAMCS). The integration of these two surveys along with the collection of personal identifiers (protected health information) allows the linking of care provided to the same patient in all departments. It is also now possible to link the survey data to the National Death Index and Medicaid and Medicare data to obtain a more complete picture of patient care. A composite measure of diabetes care effectiveness comprised of four criteria has been used to identify gaps in quality (Fig. 25.2) [27]. Recommended services include two or more hemoglobin A1c tests per year, foot exam, dilated eye exam, and flu shot. Rates are age adjusted to the 2000 US standard population using two age groups: 40–59 and 60 and over. White and Black are non-Hispanic. Hispanic includes all races. As shown here, the overall rates for compliance with the four recommended services has generally increased between 2008 and 2012 from 21 to 26.6%. The increase since 2008 for Whites represented a 33.4% improvement in compliance with recommended services, for Blacks 31.3% and for Hispanics 21.4%.

The Center for Disease Control and Prevention (CDC), with assistance from states, oversees another survey, the Behavioral Risk Factor Surveillance System (BRFSS) [28]. The BRFSS is a telephone health survey that tracks diseases and risky behaviors in the USA by calling citizens at random. Regarding lower extremities, the BRFSS surveys two process measures as part of an optional state module on diabetes: self-foot exams and foot exams by health professionals. This diabetes module has been used by approximately 40 States and territories over the past 10 years. An important characteristic of this dataset is that BRFSS results can be compared between states. The BRFSS is one of the few data monitoring tools that provides current information to track lower extremity quality process measures. There are some well-known limitations in the BRFSS approach. One limitation is that the survey might miss some of the highest risk groups such as those without residential phone listings, institutionalized patients, and unable to speak English or Spanish. Another limitation is that it relies on patient recall and perceptions, and utilizes smaller survey sample sizes to reduce costs of administering the surveys.

One of the most comprehensive and nationally representative assessments of health is the National Health and Nutrition Examination Survey (NHANES) conducted by CDC's National Center for Health Statistics [29]. NHANES includes data from a combination of patient interviews, physical examinations by physicians, and laboratory results. Diabetes-related data include survey questions, laboratory results such as fasting glucose, insulin, HbA1c and oral glucose tolerance test, lipid panel, renal function, and physical examination results including weight, height, and blood pressure. The primary advantage of NHANES as compared to patient surveys is that it includes actual laboratory and physical examination results taken on site. It is also a nationally representative sample of individuals in the United States. Disadvantages include that it is time intensive and cost prohibitive, and as such, samples sizes are relatively small. State-level data is also not available and comparisons must be made to national benchmarks.

Each decade the United States Department of Health and Human Services promotes a healthy people agenda (e.g., Healthy People 2000, 2010, 2020), which provides a framework for prevention for the health of the US population [30]. For diabetes, Healthy People 2020 strives to reach 16 objectives including five objectives to improve outcomes: reducing the incidence of diabetes and prediabetes; reducing all-cause mortality and deaths due to cardiovascular disease; reducing the rate of lower extremity amputations; and improving glycemic control. It also seeks to improve nine

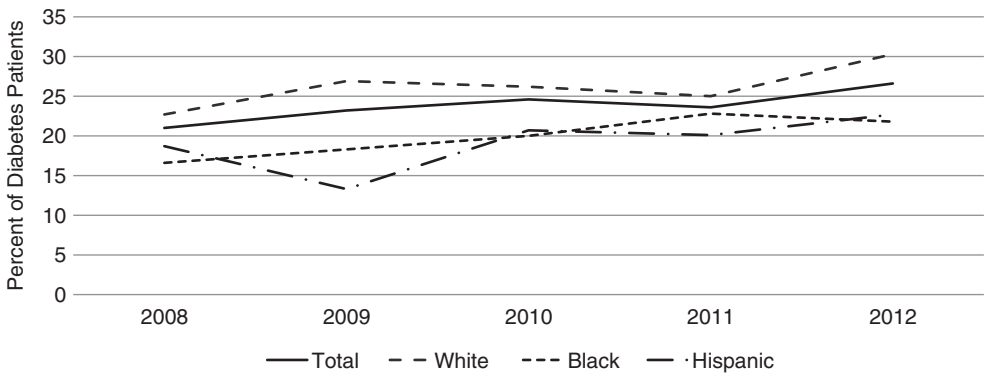

Fig. 25.2 Adults age 40 and over with diagnosed diabetes who reported receiving four recommended services for diabetes in the calendar year, by race/ethnicity, 2008–2012

areas of care relating to process measures by increasing the proportion of patients obtaining medical exams and assessments of lipids, blood pressure, HbA1c at least twice per year and dental, foot, and eye examinations. Increased detection of persons with diabetes and preventative health behaviors for prediabetes such as increased exercise, improved nutrition (less fat and calories), and weight loss are also cited as needing improvement. The HealthyPeople.gov website brings together survey data from a variety of the sources discussed above that can be abstracted by the user. Figure 25.3 displays three screenshots (a, b and c) of the query tools selecting on diabetes foot care objectives D-4 (reducing foot amputations) and D-9 (increasing foot exams). In 2010, the Healthy People goal was to reduce lower extremity amputations (LEAs) from baseline of 6.6 LEAs per 1000 people with diabetes (1997–1999 rate age-adjusted to the year 2000 standard population), to a target of 2.9 per 1000 by 2010. Data from the Healthy People 2020 midcourse review reported that the age-adjusted rate of lower extremity amputations in persons with diagnosed diabetes (Objective D-4) was 3.5 per 1000 population in 2005–2007 and 3.4 in 2008–2010 [31]. The review also reported that in 2008–2010 disparities in the age-adjusted rate of lower extremity amputations in persons with diagnosed diabetes by sex, race, and ethnicity were statistically significant. The data sources for these statistics are the National Hospital Discharge Survey (NHDS) and the National Health Interview Survey (NHIS). Between 2008 and 2010, there was little or no detectable change in the age-adjusted proportion of persons aged 18 and over with diagnosed diabetes who had annual foot examinations (68.0% and 68.4%, respectively). In 2010, disparities in the age-adjusted proportion of adults with diagnosed diabetes who had annual foot examinations (Objective D-9) by race, ethnicity, and education were statistically significant. The disparities by sex, household income, disability status, and geographic location were not statistically significant. In 2010, about 73,000 nontraumatic lower-limb amputations were performed in adults aged 20 years or older with diagnosed diabetes and approximately 60% of nontraumatic lower-limb amputations among people aged 20 years or older occur in people with diagnosed diabetes [32]. It should be noted that the reduction in the frequency of foot ulcers was originally included as a performance objective in Healthy People 2010. However, this metric was deleted because it was determined during the midcourse review that NHANES data on this topic was unreliable. This points to the fact that even within NHANES adequate quality performance data collection and measurement is difficult.

Controversy and differences of opinions exist regarding benchmarks for quality improvement in diabetes. There are several approaches for selecting quality standards and benchmarks. Most quality metrics are recommended by professional organizations. One major diabetes professional organization, American Diabetes Association (ADA), publishes its standards each year regarding high-quality care standards [33]. The section on "Foot Care" emphasizes the importance of an annual foot examination to identify foot ulcers and the screening of individuals at higher risk for ulcers and amputation including those with previous amputation, ulcer history, peripheral neuropathy, foot deformity, peripheral vascular disease, visual impairment, diabetic nephropathy, poor glycemic control, and history of cigarette smoking. The American Association of Clinical Endocrinologists (AACE) also provides clinical practice guidelines for diabetes mellitus treatment [34]. The AACE also supports annual comprehensive foot examinations which includes assessments of neuropathy and mechanical foot changes. They also recommend that patients should be referred to a podiatric surgeon, vascular surgeon, orthopedist, and/or neurologist depending on the risk factor(s) identified.

While it may be easy to identify optimal outcomes associated with best care processes based on theoretic benchmarks (i.e., no amputations, 100% foot exams), practical restrictions limit their use. A well-documented limitation is that the use of national or state averages as benchmarks are not adequate for advancing quality improvement across a wide spectrum of local diversity. Averages fail to consider severity adjustments for areas with higher-risk populations. Because of these factors, national and state averages have generally been reserved for tracking and surveillance rather than for comparative benchmarking, which requires adjustment for potential confounders. Two benchmarks currently in vogue are national consensus goals and best-in-class goals. An example of the former are the benchmarks from Healthy People national consensus goals referred to previously. There are different methods in which best-in-class benchmarks are calculated. The method of calculation determines the utility of this point of reference.

It is also important that comparisons are made with similar sources and definitions. AHRQ's National Quality Measures Clearinghouse has been a public resource for summaries of evidence-based quality measures and measure sets [35]. NQMC mission is to produce evidence to make health care safer, higher quality, more accessible, equitable, and affordable by providing objective, detailed information on quality measures, and to further their dissemination, implementation, and use to inform health care decisions. The NQMC website allows a search of all measures by general- and subclassifications such as: the type of quality measure (e.g., *outcome, patient experience, process, structure, cost, use of services, user-enrolled health state, population health state, population use of services*), organization, measure hierarchy, public health aims for quality, national quality strategy, measurement setting, current use, core quality measure, measure initiative, type of evidence supporting the cri-

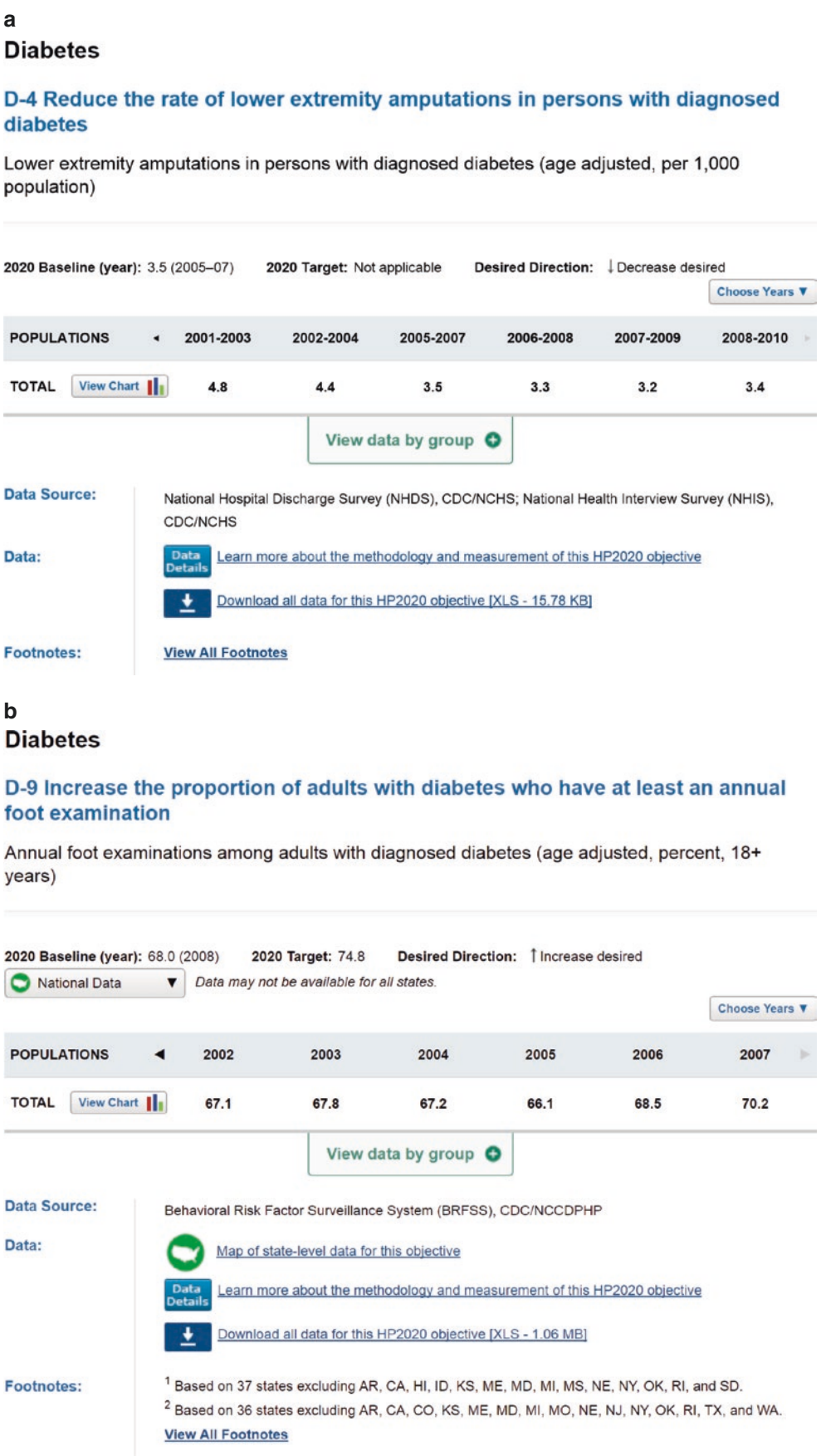

Fig. 25.3 (**a**–**c**). Examples of HealthyPeople.gov website resource detailing the HealthyPeople 2020 objectives on diabetes foot care. Available at https://www.healthypeople.gov/2020/topics-objectives/topic/diabetes/objectives. Accessed on May/1/2017

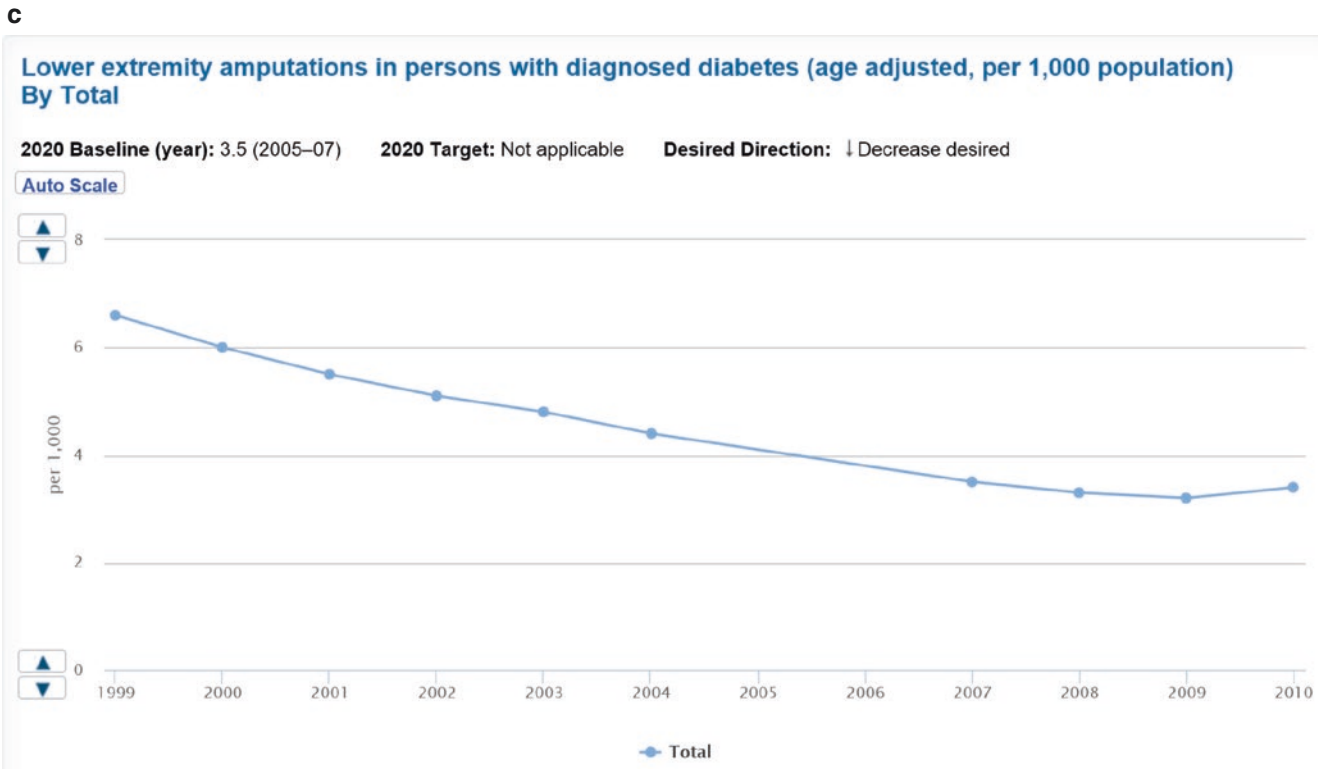

Fig. 25.3 (continued)

terion of quality for the measure, data source, target population characteristics (e.g., *age, gender, vulnerable population status),* and the Institute of Medicine Domain *(effectiveness, efficiency, equity)*. It also allows searches on user-specified terms and phrases allowing abstraction of the measure's characteristics including: Measure Domain *(Primary Measure Domain)*; Brief Abstract *(Description, Rationale, Evidence for Rationale)*; Evidence Supporting the Measure *(Type of Evidence Supporting the Criterion of Quality for the Measure, Extent of Measure Testing)*; State of Use of the Measure *(Current Use)*; Application of the Measure in its Current Use *(Measurement Setting, Professionals Involved in Delivery of Health Services, Least Aggregated Level of Services Delivery Addressed)*; Data Collection for the Measure (*Denominator Inclusions/Exclusions, Numerator Inclusions/Exclusions, Data Source)*; Computation of the Measure *(Scoring, Interpretation of Score, Allowance for Patient or Population Factors)*; and Identifying Information *(Measure Collection Name, Funding Source(s), Composition of the Group that Developed the Measure, Date of Most Current Version in NQMC)*. For example, searching on the term for "diabetes" yields a total of 340 measures that one can compare across the set of characteristics cited above, while searching on "diabetes" and "foot" brings up 14 quality measures. For illustration purposes, nine of these measures along with a small subset of their reported characteristics are given in Table 25.5.

Another group, the National Committee for Quality Assurance (NCQA), is responsible for accrediting health plans. CMS also requires health plans to report using the Health Plan Employer Data and Information Set (HEDIS). HEDIS measures are designed to allow comparisons between health care systems, although measures are also used to assess for health care quality improvement in a variety of ever-expanding ways. HEDIS measures can be obtained through the NQMC described above by searching on "HEDIS" as well from the NCQA website [36]. The information is used by health insurance companies, CMS, researchers, and other consumers to compare health care quality at several levels. For example, national magazines and media have published "America's best health plans" based on this data. Comprehensive diabetes care is among the most frequently monitored health issue that HEDIS measures. Physicians are contractually obligated to provide medical record information in a timely fashion when requested by health plans for HEDIS measures. The HEDIS measure "Comprehensive Diabetes Care" assesses adults 18–75 years of age with diabetes (type 1 and type 2) who had each of the following: (1) HbA1c testing, (2) HbA1c poor control (>9.0%), (3) HbA1c control (<8.0%), (4) and HbA1c control (<7.0%) for a selected population (commercial insurance and Medcaid), (5) Eye exam (retinal) performed, (6) Medical attention for nephropathy, and (7) BP control (<140/90 mm Hg). Currently this annually reported

Table 25.5 Select quality measures on diabetes and foot care from the National quality measures clearinghouse online search tool

Publish date	Title	Developer	Primary measure domain	Description	Measure collection name
Jun 2006	Diabetes mellitus: percent of patients who have had a comprehensive foot exam in the past 12 months.	HRSA Health Disparities Collaboratives: Diabetes Collaborative	Clinical Quality Measures: Process	This measure is used to assess the percent of diabetic patients in the clinical information system who have had an annual comprehensive foot exam documented in the last 12 months. An annual comprehensive foot exam has been part of American Diabetes Association (ADA) guidelines for some time (Lower Extremity Amputation Prevention [LEAP] exam is one type.) This examination should include assessment of protective sensation, foot structure and biomechanics, vascular status and skin integrity.	HRSA Health Disparities Collaboratives (HDC) Measures
Sep 2010	Chronic wound care: percentage of patients aged 18 years and older with a diagnosis of diabetes and foot ulcer who were prescribed an appropriate method of off-loading (pressure relief) within the 12-month reporting period.	American Society of Plastic SurgeonsNational Committee for Quality AssurancePhysician Consortium for Performance Improvement®	Clinical Quality Measures: Process	This measure is used to assess the percentage of patients aged 18 years and older with a diagnosis of diabetes and foot ulcer who were prescribed an appropriate method of off-loading (pressure relief) within the 12-month reporting period.	Chronic Wound Care Physician Performance Measurement Set
Sep 2010	Chronic wound care: percentage of patients aged 18 years and older with a diagnosis of diabetes and foot ulcer who received education regarding appropriate foot care AND daily inspection of the feet within the 12-month reporting period.	American Society of Plastic Surgeons National Committee for Quality Assurance Physician Consortium for Performance Improvement®	Clinical Quality Measures: Process	This measure is used to assess the percentage of patients aged 18 years and older with a diagnosis of diabetes and foot ulcer who received education regarding appropriate foot care AND daily inspection of the feet within the 12-month reporting period.	Chronic Wound Care Physician Performance Measurement Set
Nov 2014	Comprehensive adult diabetes care: percentage of patients 18–75 years of age with type 1 or type 2 diabetes who had a foot exam (visual inspection, a sensory exam with monofilament and a pulse exam) during the measurement year.	National Committee for Quality Assurance	Clinical Quality Measures: Process	This measure is used to assess the percentage of patients 18 to 75 years of age with type 1 or type 2 diabetes who had a foot exam (visual inspection, a sensory exam with monofilament and a pulse exam) during the measurement year. This measure is a component of the Comprehensive Adult Diabetes Care composite measure six different rates looking at how well an organization cares for the common and serious chronic disease of diabetes.	HEDIS 2015: Accountable Care Organization (ACO) Collection
Aug 2014	Diabetes mellitus: percentage of patients aged 18 years and older with a diagnosis of diabetes mellitus who had a lower extremity neurological exam performed at least once within 12 months.	American Podiatric Medical Association	Clinical Quality Measures: Process	This measure is used to assess the percentage of patients aged 18 years and older with a diagnosis of diabetes mellitus who had a lower extremity neurological exam performed at least once within 12 months.	Diabetic Foot and Ankle Care Physician Performance Measurement Set

Table 25.5 (continued)

Publish date	Title	Developer	Primary measure domain	Description	Measure collection name
Aug 2014	Diabetes mellitus: percentage of patients aged 18 years and older with a diagnosis of diabetes mellitus who were evaluated for proper footwear and sizing at least once within 12 months.	American Podiatric Medical Association	Clinical Quality Measures: Process	This measure is used to assess the percentage of patients aged 18 years and older with a diagnosis of diabetes mellitus who were evaluated for proper footwear and sizing at least once within 12 months.	Diabetic Foot and Ankle Care Physician Performance Measurement Set
May 2012	Distal symmetric polyneuropathy (DSP): percentage of patients age 18 years and older with a diagnosis of DSP who had screening tests for diabetes reviewed, requested or ordered when seen for an initial evaluation for DSP.	American Academy of Neurology	Clinical Quality Measures: Process	This measure is used to assess the percentage of patients age 18 years and older with a diagnosis of distal symmetric polyneuropathy (DSP) who had screening tests for diabetes (e.g., fasting blood sugar test, a hemoglobin A1C, or a 2 h glucose tolerance test) reviewed, requested or ordered when seen for an initial evaluation for DSP.	Distal Symmetric Polyneuropathy Quality Measurement Set
May 2012	Distal symmetric polyneuropathy (DSP): percentage of patient visits for patient age 18 years and older with a diagnosis of DSP who was queried about pain and pain interference with function using a valid and reliable instrument.	American Academy of Neurology	Clinical Quality Measures: Process	This measure is used to assess the percentage of patient visits for patient age 18 years and older with a diagnosis of distal symmetric polyneuropathy (DSP) who was queried about pain and pain interference with function using a valid and reliable instrument.	Distal Symmetric Polyneuropathy Quality Measurement Set
Mar 2015	Lower-extremity amputation among patients with diabetes: percentage of admissions for any-listed diagnosis of diabetes and any-listed procedure of lower-extremity amputation per 100,000 population, ages 18 years and older.	Agency for Healthcare Research and Quality	Related Population Health Measures: Population Use of Services	This measure is used to assess the percentage of admissions for any-listed diagnosis of diabetes and any-listed procedure of lower-extremity amputation per 100,000 population, ages 18 years and older.	Agency for Healthcare Research and Quality (AHRQ) Quality Indicators

Table 25.6 Three HEDIS diabetes care quality measures for the total population by type of insurer for the years 2005, 2010, and 2015

		Commercial		Medicaid	Medicare	
	Year	HMO	PPO	HMO	HMO	PPO
Eye exam	2005	54.8	42.7	48.6	66.5	53.8
	2010	57.7	45.5	53.1	64.6	62.3
	2015	53.7	47.1	52.7	68.8	68.4
HbA1C screen	2005	87.5	82.8	76.1	88.9	80
	2010	89.9	85.2	82.0	90.4	90.6
	2015	90.1	88.8	86.0	93.2	92.7
HbA1C > 9%	2005	29.7	55.4	49.2	23.6	27.3
	2010	27.3	46.6	44	25.9	35.2
	2015	33.8	44.3	45.4	27.4	26.5

HEDIS composite quality measure does not specifically include foot care. Table 25.6 shows three of these measures for the total population by the type of insurer for the years 2005, 2010, and 2015.

To provide clinicians with tools to support the delivery and recognition of consistent high-quality care, NCQA in partnership with the American Diabetes Association (ADA) developed the Diabetes Recognition Program (DRP) [37]. This voluntary program is designed to recognize physicians and other clinicians who use evidence-based measures and provide excellent care to their patients with diabetes. The data is analyzed, for a fee, to determine if evidence-based medicine and "excellent care" is provided, based on 10 measures from 25 patient charts. Patients and other consumers may publicly identify those physicians recognized by NCQA for providing consistent high-quality care in diabetes. There are also incentive programs encouraging eligible physicians to report data on Medicare beneficiaries. One incentive program, the Physician Quality Reporting System (PQRS) administered through CMS, ended in December 2016 and transitioned to the Merit-based Incentive Payment System (MIPS) under the CMS's Quality Payment Program [38]. The first MIPS performance period was January through December 2017. Most participants report up to six quality measures, including an outcome measure, for a minimum of 90 days and must attest to completing up to four improve-

ment activities for a minimum of 90 days. The qualifying participating clinician earns an upward payment adjustment based upon the evidence-based and practice-specific quality data submitted. There are three quality measures specific to diabetes and foot care as shown in Table 25.7. In addition to the programs described in the previous section there have been other diabetes quality improvement "Pay for Performance" diabetes programs [39, 40].

Diabetes Quality Improvement Initiatives

Since conventional health care systems in the USA are designed to provide symptom-driven responses to acute illnesses (i.e., reactionary medicine), they may not be optimally configured to meet the needs of the chronically ill. Caring for the chronically ill and population-based medicine are growing specialty areas that are particularly suited for patients with diabetes [41, 42]. The underlying principle of chronic care models as applied to diabetes is that all aspects of diabetes care are provided in a multidisciplinary setting that emphasizes proactive prevention screening practices over reactionary medicine. Health care delivery focuses on improving the community, physicians, and facilities which provide care. The chronic care model can be applied to the care of the diabetic foot by including the following essential core elements [43, 44]:

- *Organization of Care*: Preventing ulcers and amputations as an organizational priority with leadership support; establishing defined targets, evidence-based policies, and incentives to increase screening.
- *Clinical Information Systems*: Establishing registries and, if available, using electronic medical records to track patients by risk strata, giving clinicians performance feedback and risk-level-appropriate reminders for patients and providers; extracting and summarizing data from previous encounters to facilitate good clinical decision making.
- *Delivery System Design*: Providing planned visits and other proactive mechanisms for risk-stratified screening, clinical care, and follow-up in primary care; facilitating regular, meaningful coordination and interactions of foot care team members and primary care providers on basis of stratified risk level.
- *Decision Support*: Implementing evidence-based guidelines, specialist referral guidelines, and online tools; training of providers/teams; feedback and patients' progress reports.
- *Self-management Support*: Providing self-help instruction and materials to patients and families, linked to patient-identified priorities.
- *Coordination of Community Resources*: Activating patients' participation in effective community programs.
- Independent not-for-profit organizations, such as the Institute for Healthcare Improvement (IHI), have also contributed to substantial improvements in a variety of health care settings based on time-tested quality improvement tools. IHI advocates the Plan-Do-Study-Act (PDSA) model where small tested steps in change lead to desirable improvements in health care structure, process, and outcome measures (Fig. 25.4). The PDSA is guided by three fundamental questions:

1. What are we trying to accomplish? The aim must be measurable, within a specified population, and have a specific deadline.
2. How will we know that a change is an improvement? Quantitative measures are defined as a structure, process, or outcome measure.
3. What changes can we make that will result in improvement? Change doesn't always equate to improvement; changes are carefully selected.

Table 25.7 Three Merit-based Incentive Payment System (MIPS) quality measures specific to diabetes and foot care classified by AHRQ National Quality Strategy (NQS) domain

Measure name	Measure description	NQS domain	Data submission method	Primary measure Steward
Diabetes Mellitus: Diabetic Foot and Ankle Care, Peripheral Neuropathy—Neurological Evaluation	Percentage of patients aged 18 years and older with a diagnosis of diabetes mellitus who had a neurological examination of their lower extremities within 12 months	Effective Clinical Care	Registry	American Podiatric Medical Association
Diabetes Mellitus: Diabetic Foot and Ankle Care, Ulcer Prevention—Evaluation of Footwear	Percentage of patients aged 18 years and older with a diagnosis of diabetes mellitus who were evaluated for proper footwear and sizing	Effective Clinical Care	Registry	American Podiatric Medical Association
Diabetes: Foot Exam	The percentage of patients 18–75 years of age with diabetes (type 1 and type 2) who received a foot exam (visual inspection and sensory exam with mono filament and a pulse exam) during the measurement year	Effective Clinical Care	EHR	National Committee for Quality Assurance

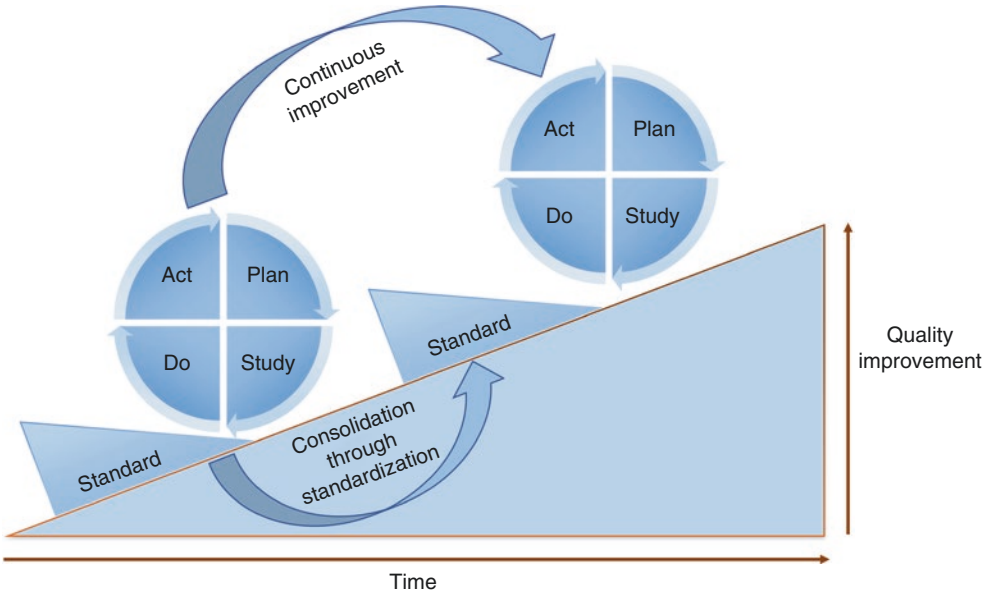

Fig. 25.4 Plan, Study, Do, Act Model for continuous quality improvement

The selected change is then put through rapid cycle testing, the PDSA model, which utilizes the information learned to guide future change. This preliminary change may either be discontinued, refined, or implemented on a larger scale or even an entire organization based on the results [31, 32].

Multiple Plan-Do-Study-Act (PDSA) cycles can be effectively utilized to drive meaningful change in all fields of health care, including diabetes. For example, the Safety Net Institute of California [45] created a collaborative with aims to improve quality of care given to persons with diabetes in several public hospital clinics. These aims focused on improved glycemic control, reduction of cardiovascular risk factors, and improvement in patient self-management. Specific goals by a designated time frame were identified, as well as structure, process, and outcome measures. Meaningful changes were selected that were believed to achieve the aims and multiple PDSA cycles were implemented. Some of the selected changes included decision support changes (uniform use of diabetes clinical guidelines, integrated communication between primary care and specialists), self-management support, delivery system design changes (specialized medical assistant training, establishing referrals to podiatry, establishing risk stratification systems, culturally appropriate educational materials, etc.), and clinical information system changes (creation of diabetes disease registry, automated alerts for overdue exams and tests, provide feedback to physicians and patients). Within 7 months, measurable improvements were seen in patient self-management by almost 50% of the patient panels, including the proportion of foot examinations (from <20 to 60%), and overall reduction in average HbA1c and LDL cholesterol levels. The collaborative realized that without strong senior leadership most initiatives are unsuccessful, information technology is critical and requires more attention, and understaffed clinics with part-time staff can struggle and suffer compared to clinics with adequate staffing and greater continuity of care.

There are many other diabetes quality improvement projects both at the state and institutional level. To improve the quality of diabetes care and outcomes and to disseminate health awareness through public relations and communication, many states have formed diabetes task forces, initiated self-management and patient education programs, supported provider training programs, developed minority and rural outreach programs, implemented information technology systems, and have established collaborations with health plans, community health centers, communities, and other agencies. State governments have recognized that to control Medicaid-related health care costs quality of care in diabetes must be addressed.

Effectiveness of Diabetes Quality Improvement Programs

The impact and level of effectiveness of the numerous quality improvement programs for diabetes is difficult to assess. Quality measures such as lower extremity amputations are long-term outcomes, and implementation of quality improvement programs cannot efficiently assess if reduction in amputations will occur in a timely manner. Instead, process measures, such as whether a foot examination was performed, are routinely monitored. The validity of the process measure is based upon the assumption of a causal relation between improvement in the process and improvement in the outcome. It is assumed that by conducting a foot-screening examination, risk factors leading to lower extremity amputation are identified and then appropriately modified, and subsequent amputation rates are reduced. The ability of this

process measure to predict outcome is subject to wide variation throughout the USA since the quality of the foot-screening exam, not just whether it has been done, is important to the validity of the causal relationship.

Even if a diabetic lower extremity screening examination is performed, the quality and components of the examination are often poorly defined and documented and may not adequately identify risk factors. This is partially due to multiple foot examination guidelines in existence, including local [46], state [47], national [48–50], and international guidelines such as the Summary Guidance for Daily Practice [51] and recommendations from ongoing research [52–54]. The foot exam can identify many risk factors; however, variations in the exam are large and little is known about current lower extremity exam strategies for gatekeepers such as general practitioners. Previous research further suggests that foot-screening examinations conducted by general practitioners are unlikely to reduce foot complications "unless they eventuate in appropriate specialist referrals" (i.e., podiatric and vascular surgeons) [55]. Appropriateness of referrals is also difficult to define, as risk factors identified may also drive the urgency to address an issue (e.g., foot ulcer, infection, Charcot neuroarthropathy).

The limited amount of time allotted to physical examinations is a significant problem for over half of general practitioners [56]. Within an annual diabetes exam, primary care providers are expected to identify and address key tests and exams including HbA1c, blood pressure, lipid panels, microalbumin measurements, serum creatinine, weight, nutrition, physical activity, medication and insulin adjustments in some, cardiovascular risk factors, dilated eye exams, other nonrelated patient concerns and conditions, as well as a comprehensive foot examination. The mean duration for a diabetes exam is approximately 15–18 min; however, the average time required for a comprehensive lower limb screening exam alone is at least 30 min. During the past decade, the number of medical problems a general practitioner addresses has significantly increased while examination time has decreased. This has resulted in less time available per problem regardless of age group, with substantial reduction in time per problem in patients 65 years of age and older [57]. By necessity, the comprehensive diabetic foot examination may be placed as a lower priority unless there are active known problems.

There are numerous other barriers that inhibit implementing the most effective prevention strategies in lower extremity complications related to diabetes. These include barriers related to patients, cultural characteristics, physician training, health plans, systems of care, societal factors, workplace, community, and the environment. Physicians in private or small group practices are unlikely to have access to the same support as those in multispecialty groups or institutions. In addition, a greater emphasis is traditionally placed on reactionary medicine (i.e., after a patient develops a foot ulcer) and more focus is needed in addressing reimbursement and health insurance coverage issues as well as physician and patient education for preventive diabetic foot screening exams. Low levels of patient compliance and education are well-known barriers, but access to primary and specialist care is also predictive of the probability of screening exams.

Finally, there are known disparities in the quality of lower extremity diabetes care. According to the National Hospital Discharge Survey, in the USA, lower extremity amputations (LEAs) from diabetes are more common among African Americans than Whites, although the age-adjusted, per 1000 rate disparity gap closed by 25% from 4.9 versus 2.4 in 2005–2007 (RR = 2.0) to 4.0 versus 2.6 (RR = 1.5) in 2008–2010 [58]. Rates also have been found to be higher among men compared to women (4.7 vs. 2.0 for 2008–2010), and lower for private versus public insurance (1.1 vs. 5.1 in 2008–2010).

Comparative Effectiveness

Comparative effectiveness research (CER) has become quite popular in recent years, especially since the American Recovery and Reinvestment Act of 2009 was signed into law. This Act originally allocated $1.1 billion to promote CER development and dissemination in health care [59]. CER compares ways in which health care conditions are diagnosed, treated, monitored, and even prevented. CER goes beyond simply comparing treatment A to treatment B, although there is great interest in comparing different treatments for a given health condition. CER also includes studying the effects of changes in the health care delivery system, and behavioral interventions [60]. Quality of life is also considered within CER, and although difficult to measure, is important when determining the true impact of a treatment on a patient [61–63].

A major limitation in assessing health care quality in the field of the diabetic limb is that most treatments provided, especially from a surgical perspective, cannot or have not undergone evaluation using controlled randomized trials. CER is even less common, making it difficult for physicians to determine treatment choices without developing a certain level of experience. Some researchers and physicians try to compare studies, either informally or through systematic reviews and meta-analyses, but small differences in the patient populations, inclusion and exclusion criteria, and methods in treatment make it difficult and sometimes impossible to draw valid comparative conclusions. Another controversial subject is whether cost should be included in a comparative analysis as this may impact the health insurance coverage of a treatment. Finally, even if CER could identify

an effective treatment, there would still be issues with overuse and underuse estimates. This is particularly true if estimates are based upon consensus guidelines instead of rigorous scientific evaluation of a treatment [64].

CER is needed to sort out the effects of numerous treatments for diabetic foot ulcers. Most treatments are compared to a standard of care with or without a control group. Standard wound care typically consists of sharp debridement, moist dressings, off-loading, addressing infection, and evaluating vascular status. The problem is that studies have shown that this standard of care usually results in only one-fourth of wounds healed after 12 weeks, and up to one-third of wounds healed by 20 weeks [65]. Clearly, there must be other methods utilized to promote faster wound healing than this currently accepted "standard of care." From hyperbaric oxygen to tissue-engineered cell-based skin equivalents, little research exists comparing similar treatments. The major challenge is that such high-quality clinical trials would be prohibitively expensive to conduct as the anticipated effect difference between two products would be much smaller than each individual product compared to the standard of care. The sample size required to detect relatively small, but clinically meaningful, effects would drive cost and time up substantially. Since most CER relies on observational data from electronic patient records, registries, and other longitudinal databases, causality cannot be fully established. There are several efforts underway to utilize electronic medical records to conduct quality assessment and reviews. For example, the FDA Sentinel system was made fully functional in February 2016, and by January 2017 had accrued health data corresponding to 223 million individuals, 425 million person-years of observation time, 43 million people currently accruing new data, 5.9 billion dispensings, 7.2 billion unique encounters, and 42 million people with >1 laboratory test result. The NIH Collaboratory Distributed Research Network [66] allows for future collaboration and data sharing to reuse and leverage the work of the Sentinel project.

Other treatments, such as surgical off-loading, may also be efficient and effective in healing and preventing recurrence of diabetic foot ulcers. Podiatric surgeons who believe in surgical off-loading provide this type of treatment because of evidence provided by a few studies, experience, and successful personal results [67, 68]. Therefore, CER and published data cannot be the only determining factor in treating diabetes foot ulcers. CER must also consider that the primary prevention of foot ulcer and prevention of ulcer recurrence are both important considerations. The recurrence of a healed ulcer is a common event, particularly when the underlying causes, such as peripheral neuropathy, calluses, increased pressure, and foot deformities, are still present [69]. Ulcer recurrence rates have been found to range from 28% at 12 months to 70–100% at 40 months [69–72]. Residual scar tissue following ulcer healing is less durable and is vulnerable to the pressures of walking [73]. Several studies suggest that the use of therapeutic shoes may be effective. For example, a London study found that the reoccurrence of ulcers among patients wearing therapeutic shoes was 17%, compared to 83% among those who returned to wearing normal shoes [74]. However, guidelines for the prescription of footwear are not standardized and few practitioners measure pressure at previous ulcer sites to ensure that high pressures are reduced by the footwear [75]. Whether all patients identified as being at risk should be provided with custom footwear is still under debate [76].

Off-loading is a key therapeutic technique essential to managing patients with neuropathic diabetic foot. It improves healing by reducing the disproportionate pressure points on the wound [77]. Prophylactic surgical off-loading and other off-loading methods may also prevent ulcer recurrence. These types of interventions aim to reduce the risk of foot ulcers by correcting deformities [78–81]. It may be determined that the presence of bony deformities and structural malalignment is too great and that the increase of ulceration or recurrence is too high. Although prophylactic surgery may provide a great benefit, long-term studies and CER is needed to demonstrate its effectiveness and surgical indications.

Health care delivery systems and behavioral intervention studies primarily address prevention of lower extremity complications that would lead to the development of diabetic foot ulcers and lower extremity amputations from diabetes. Research conducted over the past 35 years suggests that the types of health care delivery systems most likely to be successful in preventing foot ulcers and lower extremity amputations among people with diabetes are integrated, multidisciplinary teams (i.e., podiatric surgeons, general practitioners, vascular surgeons, nurses, dieticians, endocrinologists, plastic surgeons, pedorthists, infectious disease specialists, ophthalmologists and optometrists, and diabetes health educators) with risk-stratified interventions directed at patients, providers, and health care systems. A retrospective cohort study of 485 diabetic patients found that those who received podiatric care had greater survival and lower incidence of new foot ulceration than those who were not treated by a podiatric limb preservation team [82]. As wound healing and prevention of foot problems is complex, the expertise of many disciplines may be needed. The 2015 Summary Guidance for Daily Practice guidelines of the International Working Group on the Diabetic Foot (IWGDF) referenced previously states there are five key components in preventing foot problems in persons with diabetes:

- Identification of the at-risk foot
- Regular inspection and examination of the at-risk foot
- Education of patient, family, and health care providers
- Routine wearing of appropriate footwear
- Treatment of pre-ulcerative signs

Furthermore, the IWGDF stressed that LEA rates can be reduced by 49–85% through a multicomponent strategy that addresses prevention, staff and patient education, multidisciplinary treatment of foot ulcers, and close monitoring. A study of interdisciplinary preventive foot care at 10 Veteran Affairs medical centers identified six specific items that were associated with a lower rate of LEAs [83]. These were: addressing all foot care needs; making appropriate referrals; having ease in recruiting staff; having confidence with staff; availability of stand-alone, specialized, diabetic foot care services; and having providers who attended diabetic foot care education in the past 3 years. These six "must do's" for foot care in microsystems were correlated with amputation rates at a correlation coefficient greater than or equal to −0.3. One systematic review found that the evidence base to support the use of specific self-management and footwear interventions for the prevention of recurrent plantar foot ulcers was quite strong, but was small for the use of other, sometimes widely applied, interventions and was practically nonexistent for the prevention of a first foot ulcer and non-plantar foot ulcer [84].

A prospective 5-year study examined the effects of implementing the International Consensus recommendations in Pistoia (Tuscany, Italy), where all diabetic foot ulcers are seen by a multidisciplinary care team [85]. The study found that after implementation of the International Consensus, both the number and duration of hospitalizations decreased. The study identified a reduction in major amputations and a relative increase in minor amputations. Among the aspects of the program that were deemed particularly effective were its focus on the early detection of ulcers by general practitioners, a foot care education program for patients, the simple and rapid admission of patients to the hospital foot clinic, and qualified treatment by the specialist foot care team.

Patient self-management is particularly important for managing diabetes and preventing complications. Studies have demonstrated that patient self-management programs are effective tools for improving patient outcomes. One Stanford University study found that over a 2-year period participants in a chronic disease self-management program showed reductions in health distress, made fewer visits to the doctor's office and emergency room, had not experienced any further increases in disability, and had increased self-efficacy [86]. Systematic reviews of the literature on self-management programs for diabetes found positive effects on patients' knowledge, self-monitoring of blood glucose, diet, and glycemic control [87, 88]. Patient compliance is another important factor when assessing the feasibility of quality improvement programs. In one study, use of an integrated multidisciplinary team was found to lead to a low incidence of LEAs (1.1 per 1000 persons per year). However, patient adherence was a key issue. Among high-risk persons, those who missed more than 50% of their appointments with the team were 54 times more likely to develop an ulcer and 20 times more likely to require an amputation than those who kept most appointments [89]. In a more recent study, the presence of local deformity was the largest contributing factor to barefoot dynamic plantar pressure in high-risk diabetic patients and should therefore be adequately managed to reduce plantar pressure and ulcer risk. However, a significant amount of variance was still unexplained by the models, which advocates the quantitative measurement of plantar pressures in the clinical risk assessment of the patient [90]. CER must also include patient behavior to achieve its full benefit. Although patient education in foot care and foot inspection has been considered one of the most important factors in preventing amputation, relatively little research has been performed on this topic, and most studies have had a short follow-up period. Most studies have emphasized foot care and measured changes in behavior and cognition, rather than ulcer and amputation prevention. Some studies suggest that patient education improves short-term knowledge and may modestly reduce the risk of foot ulcers and amputations [91–93]. However, one study noted that the methodological quality of the nine available randomized controlled trials was poor. The review found weak evidence that patient education might reduce foot ulcers and amputations, especially among high-risk patients [94].

Research is also lacking regarding the most effective content of patient education. As noted in the International Consensus, the goal of patient education should be to increase motivation and skills, and enhance recognition of potential foot problems and taking of appropriate action. Education should be provided over multiple sessions and using a combination of methods. The ADA recommends that patients at risk should understand the implications of the loss of protective sensation, the importance of monitoring their feet daily, the proper care of the foot, and how to select appropriate footwear. Some studies support using clinical tools for enhancing patient self-monitoring. As described by Lavery et al., the incorporation of a handheld infrared skin thermometer into patients' self-care routine was found to be effective in preventing foot complications among at-risk individuals [95, 96]. The authors note that, except for traumatic wounds, areas that are likely to ulcerate often have increased temperatures due to inflammation and autolysis of tissue. Using this tool, patients assigned to the enhanced therapy group measured the temperature on the sole of the foot in the morning and evening. When elevated temperatures were found, patients were instructed to reduce their level of activity and contact a study nurse. The enhanced therapy group was found to have significantly fewer diabetic foot complications than the standard therapy patients.

CER is also needed to address the most effective interventions for physician education. The ADA recommends that all practitioners serving people with diabetes can perform a simple screening exam. Therefore, physicians may benefit

from training on best practices regarding the performance of the annual foot exam. An intervention addressing physician education is Project LEAP (Lower-Extremity Amputation Prevention) that was developed by the U.S. Department of Health and Human Services and that offers a 1-day workshop on diabetes foot care. The project has been found to improve the rate of documenting foot care education, increase self-management, and to be associated with a trend toward reduced LEAs [97, 98].

Additional systems-based and behavior-based CER is needed in lower extremity diabetic foot complications and prevention. Through randomized clinical trials, it has been shown that intensive lifestyle interventions can be more effective than medication interventions in preventing the onset of diabetes over short-term follow-up. The Diabetes Prevention Program found that lifestyle changes were just as effective as medication after 10 years of follow-up in the prevention of diabetes onset [99, 100].

Concluding Remarks

Foot ulcers and lower extremity amputations resulting from the complications of diabetes are associated with impairment of quality of life, increased morbidity and mortality and place an enormous human and financial enormous on society. While there has been much progress in the management of diabetic foot disorders over the past several decades, establishing and translating the scientific evidence into practice remains challenging. While this chapter focused on quality of care in the United States, the difficulties in achieving high quality of care for less-developed countries are magnified as much as tenfold contributing to the growing global burden of diabetes. In the United States, the lifetime risk of a diabetic patient developing a foot ulcer has been estimated to be as high as 25%. The need for preventative strategies is paramount and must be made an integral part of all future quality improvement strategies.

Using data from the National Inpatient Sample, the annual inpatient hospital cost of lower extremity foot amputations in the United States is projected to be approximately $13 billion in 2017. This does not include any postsurgical care or indirect medical costs which could double that amount. Moreover, the treatment for foot ulcers in 2017 would be $23 billion using projections from a study conducted in 2007 [101]. Diabetic foot ulcers and amputations are for the most part preventable. Multidisciplinary approaches and four podiatry visits per year were found to decrease foot ulcerations recurrence by 48%, by 53% with custom off-loading and by 73% with the use of dermal thermometer and education [102]. Lower limb amputations are typically preceded by a preventable and identifiable clinical event. It is clear that providing prevention services, reducing quality gaps, advancing scientific knowledge through comparative effectiveness research, and improving information technology capabilities will continue to play a pivotal role in improving health outcomes for persons with diabetes.

As illustrated in this chapter measuring, monitoring and analyzing the quality of care for persons with diabetes is complex and multifaceted. Federal, regional, state, and private agencies, along with physicians and researchers need to combine feasible yet meaningful and interpretable quality indicators into a standardized informational database to effectively and efficiently track changes in the quality of care. However, even more importantly quality of care programs must place a greater emphasis on the role of the patient and caregiver in managing diabetes and preventing diabetic complications. In addition to scheduling four podiatry visits per year, quality of care programs should incorporate patient and caregiver examinations using remote monitoring and telemedicine and e-health technological advances. Cellular phones can now seamlessly and reliably deliver high-resolution images, text, data, live video, and audio to clinicians and could be used in lieu of more frequent office visits. Randomized clinical trials evaluating the use of telemedicine approaches have just recently reported results or are currently in progress [102]. With camera-equipped, cell phone ownership nearly ubiquitous in 2018, technology should be incorporated into improving quality of care, increasing efficiency and reducing both direct and indirect medical costs.

Even with substantial increases in diabetes foot prevention strategies, the burden placed on specialty physicians is still great, and with the increasing prevalence of obesity will likely continue to increase. Meaningful health care systems changes are needed throughout major institutions and private practice. Strong leadership from appropriately trained physicians in health care quality is required to ensure positive and meaningful changes are implemented at both the practice and policy levels to ensure high-quality health care and improvement in population health outcomes for persons with diabetes.

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Psychosocial and Educational Implications of Diabetic Foot Complications

26

Katie Weinger, Arlene Smaldone, and Elizabeth A. Beverly

Abstract

Although interest is increasing, behavioral aspects of the diabetic foot remain emerging science. Researchers are only now beginning to investigate the psychological response to diabetic foot ulceration and amputation and the behavioral and psychological factors that influence self-care. Although cross-sectional and short-term studies have explored these areas, little long-term longitudinal data currently exist. In this chapter we review the current state of behavioral science pertaining to individuals with diabetic foot disease including barriers to prevention, precipitating factors, and therapeutic interventions. The first section describes some of the behavioral/psychological issues faced by individuals with diabetes during the course of their illness. We describe four phases of psychological responses and attempt to relate these phases to the prevention, diagnosis, or treatment of foot problems. Then, we discuss quality of life for those with peripheral neuropathy, lower extremity wounds, or amputations. Next, we discuss depression, its impact on self-care, signs and symptoms, and implications of treatment. Finally, we describe measurement instruments, strategies, and interventions that may be useful for clinicians either to incorporate into their clinical practice or as a referral for struggling patients.

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Introduction

In recent years, clinicians have begun to recognize the impact that educational, psychosocial and behavioral factors have on treatment success for leg and foot wounds. Further, many now consider quality of life an important outcome of treatment for those suffering from neuropathy, foot ulcerations, and amputations. However, although interest is increasing, behavioral aspects of the diabetic foot remain an emerging science. Researchers are only now beginning to investigate the psychological response to diabetic neuropathy, foot ulceration, and amputation and the behavioral and psychological factors that influence self-care. Although cross-sectional studies have explored these areas, few high quality longitudinal data currently exist.

In this chapter we review the current state of behavioral science pertaining to individuals with diabetic foot disease including barriers to prevention, precipitating factors, and therapeutic interventions. The first section describes some of the behavioral/psychological issues faced by individuals with diabetes during the course of their illness. We describe four phases of psychological responses and attempt to relate these phases to the prevention, diagnosis, or treatment of foot problems. Then, we discuss quality of life for those with peripheral neuropathy, lower extremity wounds, or amputations. Next, we discuss depression, its impact on self-care, signs and symptoms, and implications of treatment. Finally, we describe measurement instruments, strategies, and interventions that may be useful for clinicians either to incorporate into their clinical practice or as a referral for struggling patients.

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Phases of Psychological Responses and Educational Aspects of Diabetes

Individuals face significant events or crises at different points during the course of diabetes that challenge their usual ways of coping and dealing with stress [1–3]. These events evoke heightened anxiety, feelings of helplessness, and temporary states of cognitive confusion. People facing crisis typically employ coping strategies they have used in the past that have varying levels of effectiveness [1]. Some strategies such as denial or anger may actually interfere with health while other strategies using a more pragmatic approach serve to help incorporate information and skills into one's lifestyle [4]. Those living with diabetes face important stressors throughout the course of illness.

Four periods warrant special mention: onset of diabetes, health maintenance and prevention, early onset of complications, and the stage of illness where complications dominate [2, 3]. Each period has psychological and educational implications for the patient, family, and clinician regarding the prevention and treatment of foot problems.

Onset of Diabetes

The onset of diabetes is typically abrupt for those diagnosed with type 1 diabetes and for some diagnosed with type 2 diabetes. The patient and family, faced with the task of acquiring knowledge and "survival skills," must adapt quickly to a new and demanding regimen of insulin injections, blood glucose monitoring, nutrition, and other lifestyle adjustments. Both patients and families may experience a period of grief and mourning for loss of the healthy self and begin to adjust to the idea of living with a serious chronic illness. Prior experience with diabetes, such as having a family member or friend who has the disease, can color the response to the diagnosis. During times of crisis, individuals have difficulty both processing and retaining information [1, 5]. Yet for most people with diabetes, diagnosis is the time when they receive diabetes education, and for many people, this education is the only formal education they receive during the course of their illness. Discussion of preventive measures such as foot care are often lost or simply not addressed when faced with the priorities of acquisition of "survival skills" largely focused on the acute problems of diabetes, rather than a long-term approach to future, chronic issues.

Onset of diabetes for those diagnosed with type 2 diabetes is typically more gradual and viewed as less cumbersome. Most individuals, if worried, are more concerned about heart disease and hypertension rather than their feet. Similarly, clinicians tend to stress more immediate concerns during their initial patient interactions. Prevention of foot complications is typically not addressed nor perceived as an immediate need by patients or clinicians. In a qualitative study on patient preferences for the discussion of complications, most adults preferred the discussion of complications to occur early in the course of their diabetes rather than waiting until later in the course of diabetes, or delayed until the development of complications. They further stressed the importance of being honest but empathetic, and discussion on specific, concrete preventative steps that could help reduce the probability or severity of complications such as foot problems [6].

Maintenance of Health and Prevention of Complications

During the maintenance phase, treatment and education focus on prevention of complications, healthy lifestyle habits, and incorporating changes in lifestyle into family life. Individuals with diabetes develop diabetes "habits," self-care behaviors that can include key preventive practices such as foot care. People tend to remember and do those things that they perceive as most important, typically those instructions that clinicians particularly stressed [7]. Unfortunately, not all physicians and educators emphasize the importance of foot care. Many clinicians do not check feet at each visit [8–10] but instead may focus on glycemic control in hopes that improved glycemia will prevent foot problems [11]. Intuition and some evidence, albeit weak, suggest that preventive foot care education can improve self-care practices and decrease the incidence of ulceration and need for amputation [11–16]. Unless the physician places emphasis on foot assessment and reinforces the importance of preventative foot care during office visits, preventive foot care may be largely ignored. Both patients and clinicians need education about foot assessment [17] and preventive foot care [14, 18]. Receiving education is only a first step as it does not ensure the continued practice of self-care behaviors. Some patients may experience denial and resistance to treatment; these people typically have difficulty integrating preventive practices into their daily routines. Incorporating chronic illness into one's world-view takes time; healthcare personnel play key roles in coaching and assisting the patient to achieve this effectively. The healthcare team's reinforcement of key points and techniques is important to supporting patients' sustained self-care practices.

Early Onset of Complications

Complications of diabetes develop insidiously. Most patients go through a period of years before being affected by microvascular and macrovascular complications. Although the concept of complications should not be foreign to patients with diabetes, the onset and recognition of complications

sets a new disease trajectory affecting patients' relationships with family and providers and their self-image as a functioning, "healthy" person.

The prevalence of peripheral neuropathy is estimated to be 26–28.5% in patients with diabetes with most cases (62%) asymptomatic [19, 20]. Patients who lack protective sensation are seven times more likely to develop a foot ulcer secondary to physical and/or thermal trauma [21]. In a 2012 summary [22], treatment of peripheral vascular disease or its sequelae, foot ulceration and/or amputation, accounts for approximately 11% of $176 billion direct medical costs attributed to diabetes in the USA [22] and in 2010–2011, about 0.6% of the total healthcare costs in England [23]. Fortunately, the rate of amputation among US adults diagnosed with diabetes dropped 30% points (a 51% change) from 1990 to 2010 [24], most likely due to better glycemic status, treatment, and prevention programs.

Neuropathy is often first identified by either decreased reflexes or impaired localized sensation noted on routine examination of the feet; in most cases, both have gone unnoticed by the patient. At this point, intervention remains directed at maintaining circulation and skin integrity through heightened attention to foot care. However, for many patients, hearing this "bad news" engenders high levels of stress and anxiety that serve to block the important communication occurring between provider and patient at this time [1]. Therefore, patients may be unable to effectively process what needs to be done to maintain their health. Furthermore, people often use the experiences of others to understand their own condition. Thus, patients may use the experiences of family and friends with diabetes to frame their assumptions about complications and may assume their own course will follow a similar path. Often these assumptions are not communicated to their health provider. Yet these beliefs and assumptions about diabetic neuropathy and its management are fundamental to motivation and performance of preventive foot care behaviors [25].

As neuropathy progresses, the patient is often faced with neuropathic foot pain which may be moderate to severe in intensity and difficult to control. Treatment is often not highly effective and patients must learn to live with discomfort that impacts their usual level of activity, ability to function, and sleep. Those with painful neuropathy may respond in one of two ways: maintenance of a high level of vigilance and a renewed interest in their healthcare practices which will facilitate preventive foot care or a more fatalistic response, "there's nothing I can do to control the course of events" which will inhibit motivation to perform preventive foot care behaviors [25].

Evidence suggests that the risk of foot ulcers and their associated cost of care could be significantly reduced by appropriate screening and targeted preventive strategies geared toward good foot care [12, 14, 26, 27]. However, to be successful, these strategies must use a patient-centered approach in order to understand how patients make sense of and emotionally respond to diabetes since these are intimately linked to employment of self-care behaviors [28]. Few high quality long-term studies have examined the impact of foot education. One short-term study found that 1 h of education was not effective in reducing secondary ulcers in patients with newly healed ulcers at 6 or 12 months [27, 29]. However, longer-term studies examining the impact of more comprehensive foot ulcers prevention and treatment programs that included a multidisciplinary team were effective in preventing ulcers and other foot problems over time in low and high risk patients [27, 30–32].

Complications Dominate

Foot ulceration affects 15–30% of patients with diabetes during their lifetime [33, 34] and complications of nonhealing ulcers include infection, gangrene, and amputation of the affected limb. Foot ulcers are a causative factor in 85% of all nontraumatic lower limb amputations with resulting high morbidity and mortality [35]. Furthermore, those who undergo amputation are at higher risk to lose the remaining limb in the future [36]. Table 26.1 summarizes the psychosocial consequences of diabetic foot ulceration and amputation.

Diagnosis of a foot ulcer sets a new level of intensity to the patient's treatment regimen. Consultation with a specialist may be required for wound management. Patients will experience a double burden of illness—they still need to maintain or improve their self-care behaviors for management of diabetes but now must concurrently perform complex wound care treatment regimens, establish relationships with new clinicians, and face new implications for both long and short-term outcome. The patient may be unable to walk or drive a car, making that person dependent on others for office visits, dressing changes, obtaining treatment supplies, and routine activities of daily life. Sleep is disrupted by pain and discomfort [37, 38]. Treatment regimens are lengthy, complex, painful, and often require hospitalization. Research findings are inconsistent regarding the relationship between foot ulcer-

Table 26.1 Psychosocial consequences of foot ulcers or amputations

Reduced quality of life for both patients and caregivers
Depression
Alterations in self-image as a disabled person versus a healthy functioning person
Alterations in body image
Disruptions in family relationships
Dependency/over dependency
Alterations in social relationships
Social isolation
Sleep disturbances
Disruption in sexuality or sexual functioning

ation and psychological health. In one study of diabetic patients with foot ulcers, 68% reported a negative psychological impact that included anxiety, depression, social isolation and negative self-image [39]. In a more recent population based study [40] of adults in Scandinavia, people with diabetes and history of foot ulcers perceived their health and emotional well-being to be significantly poorer compared to those without diabetes. However, after controlling for potential confounding factors, levels of anxiety and depression and psychological well-being were similar for those with diabetes with and without foot ulcers. Negative emotions associated with ulcerations may be a response to the fear of amputation and frustration with the lengthy course of treatment and its uncertainty regarding outcome [37, 41, 42].

Adjustment to Amputation

Few studies have used longitudinal design to investigate the psychological response to amputations. Thus, we currently rely on cross-sectional data to understand factors that impact adjustment. Most of the studies that examine amputation include individuals with traumatic and medical amputations and thus are not diabetes specific. However, much of the information may apply to patients with diabetes. Phantom limb and stump pain may affect adjustment to amputation [43, 44]. Although phantom limb pain was originally viewed as psychosomatic in origin, current views hold that it also may have a physiologic basis [45, 46]. Phantom limb pain is common with one study finding 69% of persons with amputations experiencing this problem [47]. Whether psychological factors play any role in the origin of phantom limb pain is unclear. However, the presence of phantom pain may impede adjustment to amputation [44, 48] as reports have found it associated with depression [48–50], body image anxiety [48, 49, 51], and stress [43].

An individual with an amputation must cope with alterations in identity, with some viewing themselves as disabled versus healthy [43]. People with amputations will probably face the curiosity of society and the conscious or unconsciously labeling of "being different" [43–45]. These data suggest that helping individuals with a newly amputated limb prepare for societal response to their missing limb may be an important role for the healthcare team; patients need to know what to expect and anticipate how they feel and how they could respond.

Quality of Life

People value feeling well and most individuals place high priority on either maintaining or improving the way they feel. Quality of life is a multidimensional concept representing an individual's physical, emotional, and social well-being from his own unique perspective [52]. Health-related quality of life and disease-specific quality of life refer to the impact of health problems on one's everyday life: examples include the effect of disease and its treatment on a patient's functioning, health beliefs, and subjective feelings of well-being [53]. As such, health-related quality of life is subject to change over time and over the course of illness.

Neuropathy and its sequelae of foot ulceration and amputation diminish one's perception of self and feeling of wellness as these patients cope with neuropathic pain, wound management, and diminished mobility [38]. Treatment regimens for those faced with neuropathic pain are often complex [54]. The need for titration of medication dose, different medication combinations, and use of medications such as antidepressants for pain management can be difficult for patients to understand. Despite this, pain is often difficult to control. Further, treatment of foot ulceration is often burdensome, imposes additional mobility restrictions, and is of long and uncertain duration. In one study of quality of life for those with foot ulceration [55] the mean duration of ulcer treatment was 43 weeks and others have reported that approximately 70% of those receiving standard foot ulcer care will not heal after 20 weeks of treatment [56]. Although treatment duration is long, individuals with diabetic foot ulceration who seek timely care are more likely to heal today compared to those treated a decade ago [57]. However, promises of future improvement in health may not be a good motivator to follow complex treatment regimens when gains are associated with lifestyle restrictions of long duration and without guarantee of success [58].

Quality of Life and Self-Care

Clinicians need to understand the patient's perspective of quality of life in order to understand their motivation or lack of motivation for self-care including wound care. Many people with diabetes feel burdened to some degree by the rigorous demands of their disease. Rubin [52] noted that those affected by what he termed "diabetes overwhelmus" or poor quality of life often take a "to hell with it!" attitude toward their self-care, doing less than recommended to manage their diabetes resulting in diminished self-care. Thus, assessment of quality of life issues is important, because it may powerfully predict an individual's capacity to manage his disease and follow treatment recommendations.

Assessing Quality of Life

Currently no gold standard exists for the assessment of diabetes-specific quality of life and a variety of instruments have been developed and used by researchers to understand

the influence of glycemic control, treatment regimens, and complications on the person affected by diabetes. Diabetes researchers have used both general health and disease-specific quality of life instruments in order to appreciate the challenges of diabetes from the patient perspective. Diabetes quality of life studies have primarily focused on describing the health state of individuals with varying levels of symptoms and complications.

The use of intensive insulin regimens prompted interest in diabetes-specific quality of life, and thus in measuring diabetes patients' quality of life. The Diabetes Quality of Life (DQOL) [59] measure was developed for use during the Diabetes Complications and Control Trial (DCCT) and subsequently adapted for youth [60]. The DCCT found that the intensity of diabetes treatment regimen does not, in itself, impair quality of life for those treated with intensive insulin regimens [61]. The Well-Being Questionnaire [62] is another diabetes-specific quality of life measure developed for use in a World Health Organization study evaluating new treatments for the management of diabetes.

The Problem Areas in Diabetes (PAID) scale [63, 64] is both a clinical tool and an outcomes measure to identify diabetes-related emotional distress. Twenty items cover a range of emotional issues common among those with both types 1 and 2 diabetes. High scores indicate greater emotional distress and a score of greater than 40–50 merits referral to a mental health professional. Although the PAID has not been used in neuropathy studies, it strongly correlates with both depression and self-care [65] and is responsive to change over time [66], thus making it potentially useful in assessment of patients undergoing long treatment regimens such as foot ulcer therapies. Identification of individual items of concern to the patient can serve as a point of conversation during the office visit.

General health-related quality of life measures, i.e., not focusing on a specific disease such as diabetes, also provide information on quality of life in patients with diabetes. The EuroQol quality of life tool (EQ-5D) has two components: (1) a questionnaire that assesses mobility, self-care, usual activities, pain, anxiety, and depression and (2) visual analogue scale that allows patients to indicate their quality on a scale of 0 to 100 [55]. Functional health status is another important aspect of quality of life; the Short Form 36 (SF-36) is a well-used measure in this area [67]. These measures, although not diabetes specific, allow comparison of quality of life issues for those with diabetes to both the general population and those with other chronic conditions.

Researchers have employed generic quality of life instruments to study the impact of neuropathy on quality of life. Using the EQ-5D, Solli and colleagues [68] measured quality of life in a sample of 521 adults with diabetes to examine how diabetes complications influence quality of life. Controlling for potential confounders, neuropathy was independently associated with the greatest negative effect on quality of life regardless of diabetes type with mobility, pain/discomfort, and anxiety/depression most affected. In another study, Dobrota and colleagues [69] used the SF-36 to examine the contribution of neuropathic pain to quality of life in a sample of 160 adults with diabetic polyneuropathy (50% with painful neuropathy, 50% with neuropathy without pain). On average, perception of quality of life was significantly lower for those subjects where neuropathy was accompanied by pain.

Five validated instruments reflect the emerging behavioral science in this area. (Table 26.2 briefly reviews the properties of each instrument.) Three measure disease-specific quality of life (NeuroQoL [70]; PN-QOL-97 [71]; Norfolk QOL-DN [72]). A recent systematic review [73] describes each measure in detail. The remaining two instruments assess psychological predictors of foot self-care (Patient Interpretation of Neuropathy—PIN) [74] and pain (Brief Pain Inventory for Painful Diabetic Peripheral Neuropathy—BPI-DPN) [75] in patients with diabetic peripheral neuropathy. Clinicians may find these instruments useful for evaluating the clinical and psychosocial status of these patients. In addition, Turk [76] proposes five screening questions, ACT-UP interview, (Activities, Coping, Thinking, Upset, People responses), as an efficient and brief approach in the assessment of important psychosocial and behavioral issues for patients with neuropathic pain. These screening questions focus on behaviors, patient coping approaches, pain prognosis, distress, and response to pain.

Effect of Diabetes and Neuropathic Complications on Quality of Life

Only a few studies have specifically examined the effect of foot ulcers and amputation on quality of life and these have primarily used generic rather than diabetes-specific instruments. Neuropathic pain alone impacts many aspects of quality of life including sleep, physical functioning, and work status, productivity and attendance [77].

In general, quality of life is lower for those with diabetes compared to those unaffected by the disease [40, 78]. Further, quality of life for type 2 diabetes patients without complications and not on insulin was slightly higher compared to those with uncomplicated type 1 diabetes; scores for those with diabetes were similar to scores reported in other studies for adults with chronic obstructive pulmonary disease and osteoarthritis [78]. Similar findings have been reported by others [79, 80].

Complications of diabetes are the most important disease-specific determinant of quality of life for diabetes patients [81]. Quality of life is diminished not only for those affected by neuropathy and its sequelae but for their caregivers as well [64, 82]. Coffey [78] reported progressively lower

Table 26.2 Psychosocial assessment tools for patients with diabetic peripheral neuropathy

Name	Items	Scale type	Subscales/dimensions	Psychometric properties
Disease-specific quality of life				
NeuroQol [70]	43	5 point Likert scale; higher scores = poorer quality of life	Dimensions: (1) painful symptoms and paresthesis, (2) symptoms of reduced/lost feeling in the feet, (3) diffuse sensory motor symptoms, (4) limitations in daily activities, (5) interpersonal problems, (6) emotional burden, (7) overall impact of neuropathy, (8) medication side effects, (9) sleep disturbance	Validity: NeuroQoL physical symptoms associated with Neuropathy Disability Score ($P < 0.001$); Internal reliability: alpha = 0.86 to 0.95
PN-QOL-97 [71]	97	5 point Likert scale; transformed to 100 point scale; higher scores = better quality of life	16 subscales: (1) physical functioning, (2) role limitations due to physical health problems, (3) pain, (4) energy/fatigue, (5) upper extremities, (6) balance, (7) self-esteem, (8) emotional well-being, (9) stigma, (10) cognitive function, (11) role limitations due to emotional problems, (12) general health perceptions, (13) sleep, (14) social functioning, (15) sexual function, (16) health distress	Construct validity: 2 factors (physical health, mental health) supported by exploratory factor analysis Internal reliability: alpha = 0.67 to 0.97 Test-retest reliability: 0.42 to 0.84
Norfolk QOL-DN [72]	47	5 point Likert scale; higher scores = poorer quality of life	5 domains: (1) Activities of daily living, (2) symptoms, (3) small fiber, (4) large fiber, (5) autonomic	Discriminant validity: mean scores significantly higher for those with diabetes and neuropathy compared to both controls with diabetes but no neuropathy, and controls without diabetes Test-retest reliability: 0.83 to 0.94 Internal reliability: alpha = 0.6 to 0.8
Psychological predictors of foot self-care				
Patient Interpretation of Neuropathy (PIN) [74]	39	5 point Likert scale	Dimensions: (1) common sense beliefs about diabetic neuropathy (DN) and levels of understanding of DN-related medical information; (2) worry about potential consequences and anger at practitioners	Internal reliability $\acute{\alpha} = 0.62$ to 0.90; test retest reliability $r = 0.51$ to 0.64
Pain				
Brief Pain Inventory for Painful Diabetic Peripheral Neuropathy (BPI-DPN) [75]	11	10 point Likert scale with higher scores indicating greater severity or greater interference	2 subscales: Severity scale (4 items); Interference scale (7 items)	Criterion validity: moderate to strong correlation between BPI-DPN and 3 alternative pain rating scales; Discriminant validity: moderate negative correlation between BPI-DPN subscales and SF-12; identification of cut points for worst pain and average pain: mild (0–3), moderate (4–6), severe (≥7)

quality of life scores for those with symptomatic neuropathy and ulceration with the lowest scores reported for those with amputation indicating the increased health burdens presented by these complications. However, others suggest that those treated for foot ulceration may experience poorer quality of life than those with amputation because of the fear of ulcer recurrence, repeated episodes of infection and potential life-long disability [39, 83].

Findings of the Eurodiale study [84], a large multisite prospective cohort of 1008 patients presenting with new diabetic foot ulcers, adds to the body of evidence about the relationship between quality of life and its prognostic value for ulcer healing, amputation, and death. Using the EQ-5D, quality of life was measured at foot ulcer presentation; subjects were followed for 1 year or until death or amputation if sooner. Controlling for patient and ulcer characteristics and comorbidities, health-related quality of life at ulcer presentation was not associated with ulcer healing. However, major amputation and death was 31 and 37% higher respectively in subjects with lower baseline EQ-5D scores stressing the importance of assessment of health-related quality of life assessment in these patients.

Impact on Patient and Family

Qualitative studies using focus group [37] and in-depth interview [38] methodology offer insight into the experiences of

those with foot ulceration and family members who participate in their care. Foot ulcers require the incorporation of a completely different lifestyle for both patients and their caregivers and have an equally negative impact on both the patient and caregiver. Reduced mobility and diminished sense of self restricts the patient's usual life regardless of age and has consequences on sexuality and the person's ability to fulfill their role in the family or at work. Although interest in sexual activity does not diminish [85, 86], many individuals with lower extremity amputations report problems such as loss of libido and erectile dysfunction [86, 87]. Because of problems with autonomic neuropathy, sexual problems may be more prevalent among individuals with diabetes. Loss of employment is a problem for many affected by painful neuropathy, ulceration or amputation [77, 88], particularly those in occupations which require a great deal of walking or standing and is associated with reduced self-esteem especially for younger patients.

Restrictions in mobility are particularly hard for patients with diabetes who have foot ulcers. Patients are generally concerned with becoming a burden on others in terms of their daily care, shopping, cooking, and transportation to frequent medical appointments [37, 38]. Social isolation may be a problem for patients, because of the physical activity restrictions imposed by the ulcer, and for family members, because of the time and intensity burden of caring for their ill family member [37, 38]. One qualitative study [37] reports that despite their understanding that non-weight bearing would promote healing, nearly all patients could not comply either through necessity or frustration. The negative impact of foot ulceration on quality of life is pervasive for both patient and family and fraught with uncertainty about whether the ulcer will heal and, if so, whether it will recur in the future.

Implications for the Practitioner

Focusing attention on physical care of the feet without attention to the psychosocial features of health-related quality of life has important limiting effects on both patient care and strategies for intervention [38]. Greater understanding of quality of life specific to lower extremity ulcers by physicians is important to allow for improved patient-physician communication, adherence to treatment regimens and increase in patient satisfaction and quality of care. Further, assessment of the impact of diabetes on the patient is important to identify patients who may have a more difficult time in either complying with the demands of more demanding self-care regimens, or may benefit from referral to a mental health professional for counseling. The Problem Areas in Diabetes scale is particularly useful in this area.

Depression and Depressive Disorders

Depression is a serious psychiatric disorder that interferes with interpersonal relationships, quality of life, and the ability to perform and function. Both amputation and diabetes are independently associated with depression, placing these individuals at extremely high risk of depression and its consequences [89–91]. Depression may accompany amputation in the general population with older people experiencing more depression within the first 2 years following amputation and younger individuals experiencing more depression over the longer term [92].

Diabetes and Depression

The prevalence of depression for people with diabetes is about two to three times that of the general population [90, 91]. Comorbid depression occurs in all age groups, and ethnic minorities experience depressive symptoms and depression at rates that equal those of adult Caucasians [93–96]. In addition, severity of depressive symptoms is associated with poor adherence to dietary recommendations and medication regimen, functional impairment and higher healthcare costs in primary care diabetes patients [97]. High levels of diabetes-related emotional distress are associated with poor adherence to self-care behavior recommendations [65]. Thus, dysthymia, subclinical depression, and diabetes-related emotional distress can impact the success of diabetes treatment, diabetes self-care, and one's ability to care for their wound or amputation. Unfortunately, depression in diabetes is both under-recognized and, when recognized, under-treated [98–101]. Findings of a recent retrospective cohort study [102] suggest that depression is associated with a 33% higher risk of incident major lower limb amputation in veterans with diabetes and highlight the importance of screening, early diagnosis and treatment of depression.

To further complicate the picture, depression among people with diabetes is also associated with the presence of other serious complications: retinopathy, macrovascular complications of cardiovascular disease, neuropathy, nephropathy, hypertension, and sexual dysfunction [93, 103–106]. Thus, individuals with depression and peripheral vascular disease may also be coping with other serious comorbidities.

Depression may present with cognitive, physical, affective, or attitudinal symptoms. Table 26.3 lists symptoms that typically mark depression, although most people present with only some of these symptoms. The physical and cognitive symptoms often overlap with poorly controlled diabetes, making the diagnosis more difficult. Several short assessment tools such as the Beck Depression Inventory [107], the

Table 26.3 Symptoms of depression

Depressed mood	Pessimism
Loss of pleasure or interest in activities	Significant weight or appetite loss when not dieting; failure to gain age-appropriate weight
Tearfulness and crying spells	Indecisiveness
Irritability[b]	Social withdrawal or isolation
Increased sense of worthlessness or guilt	Insomnia or hypersomnia[b]
Recurrent thoughts of suicide or death[c]	Psychomotor slowing[b]
Suicide threats or attempts[c]	Psychomotor agitation
Loss of concentration[b]	
Decrease in recent memory[b]	
Fatigue; loss of energy[b]	

[a]Depressed mood and four other symptoms for over 2 weeks may indicate major depression
[b]Symptoms that may also reflect poorly controlled diabetes and/or hypoglycemia
[c]Suicidal ideation should treated as a medical emergency and assessed immediately

Hospital Anxiety and Depression Scale [108], or the Brief Symptom Inventory [109] are useful for screening for depression. Asking simple questions such as "during the past month, have you been bothered by feeling down, depressed or hopeless?" and "during the past month, have you been bothered by little interest or pleasure in doing things?" can be as successful as surveys when screening for depression [110]. If a person experiences depressed mood or loss of interest or pleasure in usual activities and at least four other depressive symptoms for a duration of at least 2 weeks, then major depression must be considered [111]. It should also be considered when these symptoms are accompanied by deterioration in glycemic control or the inability to function in the home or at work.

Treatment of Depression

Depressive disorders are usually responsive to treatment with medications or psychotherapy. Both treatments are effective used alone or in combination [103, 112]. Although the primary care provider typically initiates pharmacotherapy, knowledge of when to initiate a mental health referral is important [113]. Those with suicidal ideation are at serious risk and need immediate and appropriate referral psychiatric care. As depression improves and symptoms begin to remit, treated patients are more energetic and therefore may become at even greater risk of suicide. A mental health professional can also help (1) evaluate the success of current therapy, (2) institute combination therapy using counseling as well as medication, (3) individualize pharmacotherapy, and (4) evaluate the need for hospitalization.

Impact of Patient Education Interventions

Intuitively, diabetes foot care education integrated into a foot ulcer prevention program as well as prescription footwear, intensive podiatric care, and foot assessment and evaluation are particularly important in preventing and treating foot ulcers [114]. The evidence for diabetes education as a stand-alone intervention is weak. Multiple systematic reviews have evaluated the effectiveness of educational interventions geared at preventing foot ulcers and other foot problems concluding that there was limited robust evidence to support the effectiveness of patient education alone in preventing foot ulceration or amputation due to poor methodologic quality or lack of outcome assessment [16, 114–116]. Many of these studies included were insufficiently powered, utilized unknown or unregistered co-interventions in the control group, and/or assessed foot care knowledge and behavior via subjective outcome measures. Further, no studies examined the comparative effectiveness of education provided at different time points during the course of diabetes: at time of diagnosis, maintenance of health and complications, or at early onset of complications. Currently, there is no model of diabetes education related to complications. For adults, general diabetes education must include an introduction to specific steps to prevent all the major complications of diabetes fairly early in the course of diabetes. This initial education is not enough to last throughout the course of diabetes. Foot care must be reinforced frequently throughout the course of treatment. Health services research shows that education integrated into a multidisciplinary approach to the diabetic foot is effective for preventing ulcers and other serious foot problems [27, 30–32]. More well-done intervention studies are needed to evaluate the effectiveness of patient foot care education, preferably integrated into the foot care program, in the prevention of foot ulceration and amputation; randomized trials of treatment/prevention options should include a clearly defined education component.

Strategies to Improve Self-Care Behaviors

Patients with foot complications face challenging self-care regimens, which can lead to frustrations that interfere with glycemia and worsen outcomes [117, 118]. Therefore, patients' ability to inform clinicians about their foot care challenges, and physicians' ability to respond to patients' reports directly and in non-accusatory language are critical factors in optimizing diabetes care [119]. Depression, type 2 diabetes, and possibly neuropathy [120] are associated with cognitive dysfunction, thus the foot ulcer prevention education should be individualized and reinforced to ensure maximum benefit. Several techniques are available for use by clinicians to help

patients improve their foot care behaviors. This section describes these techniques, some of which can be easily incorporated into an office visit or other patient encounter.

Reinforcing Information

Most people remember only a small portion of the information that they receive during medical appointments. Studies that compared the information retained by patients after the appointment with the information that physicians gave patients during the appointment found that between 31 and 71% of information was forgotten [7]. Clinicians need specific techniques to reinforce important information for their patients that do not require large amounts of time and that help make the appointment more effective and efficient.

1. People tend to remember those things that are presented first, thus during the visit, discuss the most important points first.
2. Those things that are perceived as important are remembered better. Thus, when discussing a key point begin by saying: "This is very important…."
3. Simple, clear instructions are remembered better than complex or confusing instructions.
4. Be specific and concrete rather than vague. For example, "Take off your socks and check your feet and between your toes every day" is more specific and easier to follow than "Be sure to check your feet."
5. Information, particularly key points or take home messages, written down in simple terms helps reinforce learning and information retention.
6. Ask patients to prepare for their medical appointment by writing down all questions that arise during the week prior to the appointment and bring that written list to the office with them. This approach is a very efficient way to assess the patient and answer outstanding questions. People tend to remember information about issues that they have previously considered and that directly relates to them or their health.

Diabetes Education Handouts

Diabetes foot care education handouts are useful for reinforcing important self-care information and reminding patients on the specific techniques necessary to identify and prevent potential problems. As most patients are unable to recall much of received educational information during medical appointments, a handout is an effective and inexpensive way to reinforce important information. Table 26.4 provides characteristics of effective handouts.

Table 26.4 Characteristics of effective diabetes education handouts

• Simple and easily understood: Simple and easily understood information is more likely to be read and followed by the patient.
• Concise information: Concise information is more likely to be remembered.
• Portable and functional information: Easily accessible information that incorporates additional functions (e.g., calendar, wallet-size card) is less likely to be put aside and/or forgotten.
• Necessary and sufficient information: Handouts require appropriate information that promotes patients' self-care practices.
• Positive and encouraging information: Information should support patients' efforts to perform self-care, not offend or discourage them.
• Consistent information: Information provided in the handout should be consistent with other information the patient receives. For example, consistent information across education classes, one-on-one counseling, reading materials, etc.
• Good mix of text and graphics: Visually appealing information will enhance the messages contained in the handout.

Interview Techniques to Help with Patients Who Struggle with Their Self-Care

Motivational interviewing [121, 122] incorporates standard interviewing techniques in a process that is designed to help individuals who are struggling with health issues get back on track with their self-care. This technique, originally developed in the addictions field, provides a useful platform for busy clinicians to address barriers in an effective, simple manner.

Open-ended questions allow the patient to verbalize feelings and provide information in their own words thus preventing the clinician's preconceived ideas to dictate patient responses. "Tell me about…" "How are you doing with taking your medications?" "What is it like to wear the orthotic?" "What problems are you having taking care of your diabetes?"

Although questions such as "How are you doing?" and "How do you feel?" appear open-ended, they are vague and have also taken on a social context that precludes more than a superficial response of "Fine." These over-used questions do not allow the patient to adequately describe their situation or questions.

Active listening entails consciously focusing on what the person means. This skill is not as straightforward and inherent as one might expect. Although everyone listens to some extent, busy clinicians may develop a preconceived idea of what the person is feeling or attempting to express. Many people tend to think about what they will say next instead of focusing on what the patient is actually saying. Two useful tools for listening are reflection and summarizing:

(a) *Reflection*—Repeat or paraphrase statements back to the person but using the tone of a question. "You are having trouble with your exercise plan?" "You are frustrated with your treatment recommendations?"

(b) *Summarizing*—Summarizing the general idea of the patient's conversation shows that you have been listening and that you understand what the patient means. This technique also provides an opportunity to correct any misunderstandings. If the patient has outlined a plan or made other positive steps, summarizing can help reinforce their progress.

Conclusions

People with diabetes diagnosed with foot complications are at increased risk for diabetes distress and depression. Distress and depression impact the patient's ability to carry out self-care behaviors and follow through with treatment recommendations. This inability may limit the success of regimens designed to prevent and treat foot ulceration. We offer some psychosocial, communication and education strategies that can be employed by physicians and other caregivers and describe several clinical assessment tools to identify patients who are having quality of life issues and who may benefit from referral to a mental health specialist for additional counseling and/or pharmacologic intervention to help patients and family members obtain the most benefit from office visits.

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The Role of Footwear in the Prevention of Diabetic Foot Complications: The State of the Art

27

Luigi Uccioli and Claudia Giacomozzi

Abstract

Main goal of human foot evolution was to allow safe and effective barefoot motion on natural grounds. Footwear came afterwards, to protect the foot from extreme environmental conditions, to improve walking and running performance, and to cope with challenging surfaces also including new artificial substrates. But while on the one side shoes and insoles—i.e., footwear—act beneficially on the foot, on the other side they may limit foot function and render the foot itself more fragile and less prone to adaptation. This may lead the shod foot to unnaturally concentrate peak pressures under small areas at heel, forefoot, and hallux. Such a potentially dangerous condition may likely happen in diabetic patients with long-term complications, such as peripheral neuropathy, which deeply modify foot structure, foot function, and the resulting gait. Without proper protection, such patients are made vulnerable and at risk of foot ulceration. Unsuitable footwear may precipitate this condition and may be responsible for the new appearance or the recurrence of a foot ulcer. Research efforts during the last 50 years helped to better understand the criteria to take into account when prescribing or making proper footwear and/or plantar orthoses to: prevent diabetic foot ulceration (primary prevention); manage healing of an active ulcer; and prevent ulcer recurrences (secondary prevention). However, despite these huge advances in knowledge, recent literature still claims the lack of strong evidence about clinical efficacy of diabetic footwear especially in preventing first ulcer occurrence. Main reasons for that, among which the still poor adherence to treatment, are presented and discussed in the following, together with a literature review of the most recent peer-reviewed studies and position papers, to bring attention on the main causes for the still limited success of footwear intervention in diabetes and on a desirable improvement of diabetic foot care.

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Introduction

When the human foot developed into the complex biological structure we see today, with a well-defined longitudinal arch and an adducted, non-opposable hallux, it was long before footwear was invented. Main goal of this evolution was to allow humans to safely and effectively walk and run barefoot on natural substrates. Since then, foot bones arrangement experienced only minor changes, being already optimized to interact with gravity and earth environment. Footwear came long later, to protect the foot from extreme environmental conditions, to improve walking and running performance, to cope with challenging surfaces also including new artificial substrates. Fashion soon became another relevant issue to take into account, especially in industrialized countries, with a dramatic impact on shoe design. Thus, while on the one side shoes and insoles act beneficially on the foot–ankle complex, on the other side they negatively interfere with both foot structure and function and render the foot itself more fragile and less prone to adaptation. It has been showed, in fact, that the natural unshod foot is longer and wider than the shod one under comparable height and BMI. And while the former more uniformly redistributes load on a broad surface, the latter unnaturally concentrates peak pressures under smaller areas like heel, forefoot and hallux. Thus, even in the presence of a non-pathological condition, the prolonged use of unsuitable and uncomfortable footwear may represent the main cause for the onset of relevant and potentially dangerous changes in gait biomechanics. This is especially true for diabetic patients with long-term complications, such as peripheral neuropathy, which deeply modify foot structure, foot function, and the resulting gait. Without proper

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protection, such patients are made vulnerable and at risk of foot ulceration. Unsuitable footwear may precipitate this condition and may be responsible for the appearance of a foot ulcer. Even worse, once the ulceration process has started, avoiding ulcer recurrences is indeed a challenge.

Researchers have been working for more than 50 years at a worldwide level to gain and share knowledge in the field of diabetic foot care. These efforts helped to better understand the criteria which should be taken in mind when prescribing or making proper footwear and/or plantar orthoses to prevent diabetic foot ulceration (primary prevention), to manage healing of an active ulcer, or to prevent ulcer recurrences (secondary prevention). However, despite these huge advances in knowledge, recent literature still claims the lack of strong evidence about clinical efficacy of diabetic footwear especially in preventing first ulcer occurrence. Main reasons for that, among which the still poor adherence to treatment, will be presented and discussed in the following.

Here below, a short overview of the main effects of diabetes and diabetic complications is reported, with a special attention to the role of these changes in the altered foot biomechanics. The agreed criteria and solutions to prescribe proper footwear are then summarized with respect to all classes of ulcer risk. Some new ideas and proposals are also discussed. Finally, a literature review of the most recent peer-reviewed studies is reported, to bring attention to the main causes for the still limited success of footwear intervention in diabetes.

The Effect of Diabetes on the Main Structures of the Foot–Ankle Complex

Diabetes, and diabetes complications, is responsible for progressive changes in foot structure and function. Changes occur at the level of almost all tissues of the foot–ankle complex, in different combination according to the specific patient's clinical status and morphology [1–5].

Effects on tendons and ligaments: due to protein glycosylation and the consequent collagen abnormalities, tendons and ligaments show greater transversal sections than usual; the thickening, which increases concurrently with the severity of the disease, in particular with the increase of disease duration and the worsening of metabolic control, contributes to the greater stiffness of these tissues. This process is particularly evident in plantar aponeurosis (plantar fascia) and Achilles tendon, whose reduced elasticity significantly limits the physiological performance of gait.

Effects on cartilage: the characteristics of healthy cartilage result in an essential help to gait and standing, i.e., range of motion of each foot and ankle joint is easily maintained due to well-lubricated bone interfaces, and an accommodative action is done in standing which helps to maintain balance of leg bones over the talus with a minimum involvement of muscular structures. Similarly to tendons and ligaments, diabetic foot cartilage changes its composition mainly due to the modification of collagen fibers; this increases its stiffness and represents an obstacle in the performance of physiological range of motion of each and every foot and ankle joint.

Effects on muscles: diabetes mellitus entails a severe damage to nerve conduction, thus causing a worsening in the management of the related muscle fibers; as a consequence, both intrinsic and extrinsic muscles of the foot–ankle complex are damaged as for structure (reduction of muscle volume) and function (reduction of muscle strength); more specifically, a reduced function of tibialis anterior leads to a poor control of foot landing at heel strike and of toe clearance at toe-off, while a reduced function of intrinsic muscles entails a poor stabilization of foot bones and arches during loading acceptance and propulsion and a significant unbalance between muscle and ligament action during the entire stance phase.

Effects on peripheral sensory system: peripheral neuropathy is often associated with long duration of diabetic disease and with unstable metabolic control. Symmetrically present at the distal part of both lower and upper limbs, it significantly impacts on the peripheral sensory system. As a consequence at the lower limbs, patients experience a certain loss of protective sensation under the sole as well as on the dorsum of the foot. This exposes the foot to risk of damage for thermal or mechanical reasons, and to the late detection of tissue breakdown and infection processes.

Effects on skin: the skin, and the soft tissues immediately underneath the skin of a diabetic foot which experiences the above alterations and the below-described deformities, are stressed by compressive loading greater than normal, as well as by shear forces higher than usual both under the foot sole and on the dorsum of the foot. The abnormal load related to both vertical forces and shear stress may induce tissue damage starting from the inner part of the soft tissue, explaining from a mechanically point of view why the onset of ulceration processes is so deeply related to traumatic tissue damage. Besides this, the skin of the diabetic foot, even in the absence of the other discussed alterations, suffers from loss of autonomic control and from a consequent reduced hydration, which renders it less elastic and thus more vulnerable to the action of increased mechanical stress.

Effects on foot morphology (deformities): due to most of the above alterations, first of all to the significant muscle unbalance and atrophy and to the increased stiffness of tendons and ligaments, the diabetic foot undergoes serious alterations of its morphology. Most common deformities of the diabetic foot are represented by an excessively high longitudinal arch (rigid cavus foot), hammer toes, and hallux valgus. Foot deformities are responsible for the forward shift of the submetatarsal adipose pads, following which the metatarsal heads come into direct contact with the ground [6]. The consequent development of hyperkeratosis as a

response mechanism to the overload, is in itself responsible for further increase of local pressure (indeed, it has been ascertained that the removal of a hyperkeratosis is able to reduce local peak pressure up to 30%) [7]. The final result of the overall alteration process is a rigid cavus foot, less adaptable to the floor during the foot–floor interaction; it remains rigid during the whole walking cycle with few exposed small areas of contact, thus originating high plantar pressures. A relationship between plantar fascia thickness and forefoot increased vertical forces has been found through experimental measurements, thus supporting the hypothesis that soft tissue abnormalities may contribute to the development of a different, more demanding pattern of pressure distribution under the foot [8]. Some prospective studies have also demonstrated the relationship between areas of high pressure and the subsequent development of ulcers [9]. It should also be observed that an increase in local peak pressure associated with insensitivity represents an increased risk for tissue damage; indeed, patients with rheumatoid arthritis with comparable peak pressures do not experience ulceration, likely due to the protective sensation of pain which stimulates the patient to offload the painful area [10]. Therefore, it is fairly evident that a reduction in local peak pressure, as is the aim of diabetic shoes and insoles, represents a potentially effective means of reducing the risk of ulceration. Finally, in the presence of Charcot Neuroarthropathy, foot deformities become so severe that they may require strategic, personalized treatment also including temporary foot offloading.

The role of patient's weight. An additional diabetes-related damaging factor is represented by the weight of the patient. Clinical practice confirms that a high percentage of long-term type II Diabetic patients is overweight or obese. In these patients, the excessive load may have an expected negative impact on foot structures, especially on feet with already misaligned lines of force resulting from the described deformities. Further, to cope with gravity and maintain gait effective, obese people reasonably develop a more supinated foot, with expected, very high pressures at the forefoot and hallux; this alteration may even worsen and accelerate the development of the typical diabetic neuropathic foot.

As a last comment, it is worth to add here the observation that, in several cases, comorbidities or preexistent neurological or orthopedic diseases further compromise the global foot structure and function, thus rendering the effects of diabetes even more difficult to cope with.

Proper Footwear: Special Needs for Diabetic Patients

The described damages to the various foot structures become more and more evident as the long-term complications, such as sensory and motor neuropathy, are fully developed. However, all potential effects and changes should be taken into account even at a mild level of complications, when the patient still does not have the need for a specific custom-made footwear prescription. In early, mild-level stages of peripheral neuropathy, in fact, as much as possible should be done not only to cope with, but also to prevent or delay the onset of biomechanical alterations. The choice of a proper footwear, together with a strong education program, can be effective in addressing such issues. Several studies support the belief that inappropriate footwear causes ulceration. It is fairly obvious that given their vulnerability, diabetic subjects must select footwear which does not pose a further threat of risk and which ideally should serve as a form of protection. It is important that the physician, conscious of the importance of the role of footwear, be fully informed in order to make suitable recommendations. In turn, the patient must be made aware of the potential risk of lesion posed by unsuitable footwear, and must be encouraged to accept selecting a certain type of footwear which may not necessarily coincide with personal taste. At a late stance, when patients are seriously compromised, and the prevention of ulcer formation or recurrence is of primary importance, main function asked to proper footwear is surely accommodative and protective rather than corrective. Independently from the level of peripheral neuropathy, key common elements for the choice of proper footwear are: (a) redistribution of plantar pressure in order to avoid high localized peaks; (b) reduction of friction; (c) prevention of mechanical and thermal traumas; and (d) restoration or maintaining of foot function during gait. A certain consensus has been reached on the main criteria for footwear design and prescription according to the presence and severity of the diabetic neuropathy.

Categories of Risk and Footwear Recommendations

Not all patients have the same level of risk to develop foot ulcers, and a number of factors, including the presence/absence of protective sense perception, presence/absence of significant foot deformities, presence/absence of previous ulcers, and eventual simultaneous presence of additional complications or comorbidities, should be evaluated when determining risk categories and planning corrective means of prevention [11].

Literature reports about various scales and methods to classify diabetic feet according to the risk of ulceration. A 2005 valuable Review paper by Singh et al. [12] identified the following summary of risk classifications from Professional Organizations on Diabetic foot screening (Table 27.1):

Based on the most recent IWGDF Guidelines, delivered in 2015 [13], the remaining part of this paragraph deal with

Table 27.1 Diabetic foot screening: summary of main risk classifications from Professional Organizations [12]

Organization	Risk category	Description
International Working Group on the Diabetic Foot (IWGDF)	0	No sensory neuropathy
	1	Sensory neuropathy only
	2	Sensory neuropathy plus peripheral vascular disease and/or foot deformities
	3	Previous foot ulcer
American Diabetes Association	No risk	No risk factors for foot ulcer
	High risk	Peripheral neuropathy, altered biomechanics, increased pressure, bony deformity, peripheral vascular disease, history of foot ulcer or amputation, or severe nail pathology
US Veterans Health Agency and Department of Defense	High risk	Lack of protective sensation, peripheral vascular disease, foot deformities, history of foot ulcer or non traumatic amputation
American College of Foot and Ankle Surgeons		No universally accepted system, but includes International Working Group's categorization
Collaborative Group From the United Kingdom (the Royal College of General Practitioners, the British Diabetic Association, the Royal College of Physicians, and the Royal College of Nursing)	Low risk	Normal sensation, palpable pulses
	At risk	Neuropathy, absent pedal pulses, or other risk factor
	High risk	Risk factor plus foot deformity, skin changes, or previous ulcer

Table 27.2 The IWGDF Risk Classification System 2015 and preventative screening frequency [13]

Category	Characteristics	Frequency
0	No peripheral neuropathy	Once a year
1	Peripheral neuropathy	Once every 6 months
2	Peripheral neuropathy with peripheral artery disease and/or a foot deformity	Once every 3–6 months
3	Peripheral neuropathy and a history of foot ulcer or lower-extremity amputation	Once every 1–3 months

the four risk categories IWGDF identified on the basis of the above criteria (Table 27.2).

Category 0 (No peripheral neuropathy): Patients Not at Risk of Ulceration (Primary Prevention). These patients do not have active or previous lesions, nor chronic complications, and maintain a protective sensation since they are not affected by peripheral neuropathy. They require adequate education, but not a substantial change with respect to footwear for daily use, unless they are using too tight, too high-heeled shoes or, in general, shoes which are not compliant with their health status, habits and behavior, and daily physical activity. In general terms, given their diabetic status, they should be simply encouraged to evaluate a number of factors when selecting footwear, most important of which whether the shoe is well-fitting. It must be kept in mind, in fact, that too narrow shoes act with high friction on the foot skin, both on the plantar surface and on dorsum, but too large or too long shoes entail high friction as well, due to the undesired relative movement between the foot and the shoe. Patients should thus avoid tight-fitting footwear with narrow forefoot, tight toe box or tight instep, while they should look for comfortably shaped shoes with soft uppers and a sole able to absorb excessive spikes of vertical forces (Fig. 27.1). Shoes marketed with different widths for each size should be preferred in order to better fit the natural shape of the foot without constraints. Custom-made inserts are usually not necessary to these patients. Education on selection of suitable footwear is very important. The practice should be encouraged of measuring both feet—which are frequently quite different in length—and of doing that while the feet are "at rest," i.e., they are not stressed by fatiguing activities. Table 27.3 shows some basic suggestions for the correct selection of footwear. Foot deformities may be present in some of these patients due to preexistent orthopedic or neurological diseases, or as a consequence of overweight or obesity. In this case, special care should be paid in recommending the proper footwear so as to get the maximum compliance with the deformities and/or the increased volume of the foot. Even though recommended frequency of reevaluation is once per year, in the presence of some preexistent deformities a higher rate of reevaluation might be more appropriate.

Category 1 (Peripheral neuropathy): Patients at Medium Risk of Ulceration (Primary Prevention). These patients

Fig. 27.1 Shoes with soft sole and amply shaped soft upper available in different widths suitable for risk classes 0 and 1

Table 27.3 Recommendations for an appropriate, safe selection of footwear

	Description
R1	Both feet should be measured with an appropriate measuring device
R2	Feet should be measured *at rest*, i.e., not after prolonged and fatiguing activities
R3	Both shoes should be fit while standing
R4	The position at the first metatarsophalangeal joint should be checked. It should be located in the widest portion of the shoe
R5	The right length of the shoe should be checked; additional volume should be considered at the top of the toes. An additional length between 3/8 and 1/2″ should remain between the end of the shoe and the longest toe
R6	The proper width should be tested; enough space should be present around the ball of the foot. A soft and moldable upper with extra space should be selected in the presence of foot deformities
R7	Shoes should include a firm heel counter for rearfoot stability with a soft padded collar
R8	Shoes with laces or straps should be selected because they allow a wider open and an easier entry into the shoes; they also allow a better fitting with the foot shape

Fig. 27.2 Oxford, soft flexible leather laced shoes of adequate size to accommodate pressure—dissipating accommodative insoles

Fig. 27.3 Single layer, pressure—dissipating accommodative Insole

experience a sensory neuropathy with an ensuing loss of protective sensation and a subsequent risk of ulceration. Education to a proper foot care and a proper footwear selection is extremely important for these patients. First of all, they must be convinced to avoid walking barefoot and wearing mended socks, and learn to substitute the loss of sensation with alternative senses (e.g., eyesight or hand touch). When sensation is lost, thermal damages may occur as well as mechanical traumas, thus patients must learn to sample water temperature by hand before washing their feet in order to avoid burns, to detect foreign bodies, such as pebbles, before putting on their shoes, and to evaluate other dangerous signs (e.g., tacks or worn soles). Even if no strong evidence has been proven up to now with respect to the efficacy of footwear in the primary prevention of ulcers, patients, as well as relatives and caregivers, should be well aware that the selection of footwear cannot be no longer based on the usual criteria of immediate sensation of comfort. Indeed, in the presence of sensory neuropathy, the patient might perceive even tight shoes as comfortable. Therefore, it is essential that both feet are measured in all their dimensions and that the footwear contain the foot without even the minimum constraint. Education in the selection of the shoes is therefore very important in this category (Table 27.3). Soft leather laced shoes of adequate size to accommodate the foot volume and properly redistribute forces—coupled with dissipating accommodative insoles—should be preferred (Figs. 27.2 and 27.3). Shoes and inserts should however allow as much as possible the maintenance of a physiological gait [14] and of proper mobility and function of foot and ankle joints.

Category 2 (Peripheral neuropathy with peripheral artery disease and/or a foot deformity): Patients at High Risk of Ulceration (Primary Prevention). When the loss of protective sensation is complicated by foot deformities (e.g., bunion, claw toe, hammer toe), whether independent of diabetes (e.g., idiopathic bunion) or more frequently secondary to motor neuropathy, the risk of ulceration is considerably increased [15]. In those cases where foot deformities (e.g., toes deformities) are accommodated in unsuitable footwear, the mechanism underlying the lesion involves friction, most frequently caused by the upper part of the shoe, which in the first instance determines a superficial abrasion and later an outright ulcer. Ulcers associated with this sort of friction are usually localized on the top of the toes and on the lateral surfaces of the first and fifth toe. According to Eurodiale data, toes are the most frequent place where the ulcers do develop [16]. These cases neces-

sitate an even greater awareness of the correct selection of footwear both in terms of shape, as the foot should not be constrained in any way, and from the point of view of the materials used, especially for the uppers. These should be made of soft and flexible material, adaptable to any surface irregularities so as to guarantee a perfect fitting and to avoid the threat of friction. For the same reasons, stitching inside the shoe must be avoided as well. Nonetheless, the increased risk associated with foot deformities is not exclusively due to the difficulty in accommodating deformed toes, but also to the complex management of the biomechanical changes in gait pattern associated with such deformities. Among the most evident and most important changes, it is worth to note: poor control of landing and propulsion due to a weaker tibialis anterior; loss of stability due to atrophy of the lumbrical and interosseous muscles [17]; forward shifting of the metatarsal head pads with exposure of metatarsal heads due to unbalance between muscle and ligaments stabilization action; reduction of joint mobility at the foot and ankle joints; alteration in the walking pattern which moves from an ankle-based to a hip-based walking strategy [18]; appearance and persistence of overload at the metatarsal level in the propulsion and toe-off phases due to most of the above effects [19, 20]. Patients in this category benefit greatly from the use of footwear which, while accommodate structural deformities, at the same time cope with and try to mitigate as many as possible of the above biomechanical defects [21]. Main footwear recommendations are summarized here below:

- Shoes with "biomechanical properties" allow the adoption of a protected walking pattern, with reduced peak plantar pressures and a roll-off process which avoids excessively prolonged loading of at-risk areas. This may be guaranteed by a total contact between the foot and the internal part of the shoe—usually obtained by custom accommodative foot orthoses—along the entire stance phase, and by using specific rocker bottom soles that allow an adequate impact at heel strike and—through a pivot point inserted immediately behind the metatarsal heads or, in any case, placed just proximal to the area in which pressure relief is needed—a smooth transition from midstance to propulsion; finally, a wide angle between the sole and the ground at the most anterior part of the shoe further reduces the stress at the level of metatarsal heads during propulsion and toe-off (Fig. 27.4) [22].
- Many off-the-shelf walking and running shoes are currently equipped with a mild rocker sole which is quite effective at reducing plantar pressures in the forefoot and at delivering some metatarsal head relief and gait assistance; however, the pivot point might not be properly placed and the flexible sole might not be effective in redistributing forces for a diabetic, high risky foot.
- Shoes should always be extra-depth shoes, so as to accommodate custom-made total contact inserts (TCIs).
- When the main issue to be addressed is the reduction of peak plantar pressures—i.e., the patient is not yet compromised as for other biomechanical aspects like poor joint mobility or muscle performance—some running shoes designed for maximal forefoot pressure relief may be effective in decreasing plantar pressure, especially when used in conjunction with viscoelastic insoles (Fig. 27.5); it must however be noticed again, as for the above-cited memory foam, that viscous materials, while representing excellent dampers to absorb shocks and high load, partly dissipate the energy that is instead released by proper elastic material during propulsion; thus, they should be used with care in case patients show a weak propulsion. In general, then, solutions involving very soft materials should be carefully assessed with respect to the

Fig. 27.4 Section of a shoe with "biomechanical properties": the recessed heel allows a soft impact at heel strike; the point of rolling inserted immediately behind the metatarsal heads allows a smooth transition from midstance to propulsion; the presence of a wider angle between the sole and the ground at the most anterior part of the shoe further reduces the stress at the level of metatarsal heads during propulsion and toe-off

Fig. 27.5 Total contact, custom-fabricated, pressure-dissipating accommodative foot orthoses

patient's habits and anthropometry, i.e., they will likely decrease their performance quickly in the presence of overweight, obese, and/or quite active patients.

- A rigid sole better reduces forefoot pressures when compared with a flexible sole, since it maximizes foot contact area especially during late stance phase. When designing this kind of footwear, it is important to consider the position of the pivot point (rolling point of the step): a pivot point placed immediately behind the metatarsal heads usually guarantees for a reduction of peak pressure up to 30% (Fig. 27.6), with a potential further 20% of reduction made possible by the materials and the number of layers of customized inserts (Fig. 27.7).
- Finally, there is a need for an appropriate loading relief by means of total contact foot orthoses which are customized, able to dissipate compressive load (i.e., pressure) and to accommodate the foot and its deformities; the insoles will likely be inserted in deep lacing shoes manufactured in soft leather, without dangerous stitching, with a frontal region designed to suitably accommodate claw or hammer toes.

All the above recommendations are even more important when patients are further complicated by peripheral artery disease, an aspect which alone entails an even greater attention to undesired compression of the whole foot and ankle complex. This last aspect, in fact, should be always kept in mind when treating category 2 patients even in the presence of only negligible foot deformities.

Category 3 (Peripheral neuropathy and a history of foot ulcer or lower-extremity amputation): Already ulcerated patients, at Very High Risk of Ulceration (Secondary Prevention). This category includes patients who have already had an ulcer which has healed. Treatment during ulcer healing is discussed in the following paragraphs. Diabetic patients with a history of relapsing plantar ulcers or patients with a previous minor amputation have abnormally elevated pressures under their feet, partly due to the modified mechanical properties of the new, cicatrized tissues. Peak pressures most often occur under the metatarsal heads and correlate with sites of ulceration, and the risk of relapse is high indeed, up to 50% in a year. The reduction of peak pressure through the use of appropriate footwear and plantar orthoses, as well as the restoration of a safe gait and the protection of the foot or the residual foot represent very important aspects in an effective program of secondary prevention of the neuropathic foot (Figs. 27.7 and 27.8). Further, it is here worth to remark the special attention and care the contralateral foot asks for: while 1 foot had been experiencing the ulceration and healing process, in fact, the contralateral foot had simultaneously been supporting a higher stress than that expected during a symmetric, balanced gait. Thus, being the "healthy" foot equally fragile as the ulcerated one due to the general clinical status of the patient, its risk of ulceration might be very high indeed. In terms of recommendations for the selection of footwear for this group of patients, the principles outlined for category 2 patients hold true for this category as well. In particular, patients should be encouraged to select footwear with rigid rocker soles and molded inserts, preferably multilayered insofar as this type seems most beneficial in reducing peak pressures. In contrast to primary

Fig. 27.6 Footwear with a rigid rocker sole. The rigid sole minimizes the metatarsal–phalangeal joint articulation tension and maximizes foot contact area during late stance phase

Fig. 27.7 Total contact inserts can reduce pressure peaks under the foot by maximizing the contact area and spreading the pressure over a larger plantar surface

Fig. 27.8 Shoe with a rigid rocker sole and very high toe box to content deformed toes and multilayered customized insole

prevention, various studies have demonstrated the protective effect of footwear in secondary prevention and a consequent reduction of rate of relapse, both in terms of bespoke solutions and prefabricated commercially designed models (even though it is quite common that, due to specific deformities, there is the need for custom-made solutions). Among advantages and drawbacks of bespoke solutions with respect to prefabricated models, it is worth to mention the better fitting and the optimization of the accommodative and therapeutic solutions (advantages), as well as the higher cost, the longer delivery time and the limited adaptation to fashion (drawbacks). Researchers and clinicians are currently working on a clear demonstration of the evidence of the intervention effectiveness, as better discussed in the recent review section of this chapter. The issue is quite complex since an adequate level of treatment standardization has still to be reached. As a couple of examples, The Consensus Development Conference on Diabetic Foot Wound Care generically reported that "Footwear should be prescribed, manufactured, and dispensed by individuals with experience in the care of diabetic foot" [23]. Current Medicare guidelines, related to the Therapeutic Shoe Bill (TSB), give indications about the type of footwear and plantar orthoses which can be prescribed and reimbursed in case of diabetic foot pathologies. Basically, the TSB Medicare (Part B) [24] states that a Diabetic patient may receive annually Medicare reimbursement for one pair of adjustable extra-depth shoes and three pairs of multidensity inserts, or one pair of custom-molded shoes with inserts and two additional pairs of inserts. Medicare covers shoe modifications instead of inserts. All people with Part B who have diabetes and severe diabetic foot disease are covered. The medical doctor must certify that the patient needs therapeutic shoes or inserts. A podiatrist or other qualified doctor must prescribe these items and they must be provided by one of the following professionals: a podiatrist, an orthotist, a prosthetist, a pedorthist, or another qualified individual (from https://www.medicare.gov/coverage, last check November 2016).

Specific needs are required for category 3 patients in the Presence of Partial Amputation [22]. In this case, it is important to work to restore stability and function, to facilitate an energy-efficient gait, to maintain balance, and to prevent further complications. To reach these goals, it is essential to provide appropriate footwear and custom-made foot orthoses or prostheses. Basically, the criteria explained and reported here above also apply to this kind of foot, even though some peculiarities must be taken into account, like the altered foot proportions, eventual change of volume consequent to the surgical intervention, the loss of the propulsive lever represented by metatarsals and toes. Just for this reason, in a partially amputated foot, the solution of the rigid rocker sole with a proper pivot point proximal to the amputation and an adequate forefoot angle is a reasonable way to allow center of pressure to progress forward, anteriorly with respect to the distal end of the residual foot. Abnormal plantar pressure and shear force can be addressed and relieved with custom foot orthoses. Lower limb orthoses or ankle foot orthoses (AFOs) and prostheses may help restore functional gait; more specifically, AFOs can be utilized to replace the lost lever arm of a transmetatarsal or hallux amputation: usually, a special insole is suggested containing an extended spring shank made of steel or carbon graphite composite (lighter but less robust). The shank keeps the shoe from bending, thus reducing forces through midfoot and forefoot (continuity of rocker sole). Partial foot prostheses, even though receiving great acceptance from the patients, needs to be used cautiously in patients with diabetes because of the presence of peripheral artery disease and/or neuropathy: usually, they consist of partial foot prostheses made of silicone or acrylic resin (i.e., Chicago boot or a Lange prosthesis) which show good cushioning, stability, and excellent absorption of shear forces. Not to be underestimated, these prostheses often result to be cosmetically pleasant. As major drawbacks, they present some difficulties to be put on and off, they tend to be hot and not to permit air circulation and to macerate the skin, while allowing the possible growth of bacteria. Recent technology innovation based on 3D modeling and printing seems especially promising in greatly improving personalization and optimization of such prostheses while, at the same time, containing costs and render them accessible to a much wider population. Of course, a lot of research, development and validation work is still needed; however, some preliminary experiences encourage the exploration of this new scenario (Fig. 27.9). Shoes should be easily modifiable: soles made of EVA (ethylene vinyl acetate), neoprene or injection-molded polyurethane are easy to be worked; leather sole shoes are not difficult to modify but can become heavy and cumbersome when modified adding lifts, shanks, or rocker soles; rubber sole shoes are not easy to modify. Equally difficult to modify and adapt are those shoes with extraordinary shock-absorbing features like air bladders, pockets of gel, or springs. As for each neuropathic patient, the upper portion of the shoe should be made of material which is moldable, stretchable, without internal stitching, and breathable like leather. The interior lining should be made of supple leather. Useful are also lining materials that wick moisture away from the skin, such as Gore-Tex, or have antibacterial properties. High top shoes tend to work well for patients with transmetatarsal, Lisfranc, and Chopart amputations. Blucher opening should be preferred to a balmoral opening (adjustability and space across the instep and forefoot areas). A lace-to-toe or surgical opening might be preferred but usually not well accepted. Slip-on shoes should be avoided as most are tight and restricting, and do not cover enough of the dorsum of the foot. For the partial amputated foot, a custom-made short shoe may be more

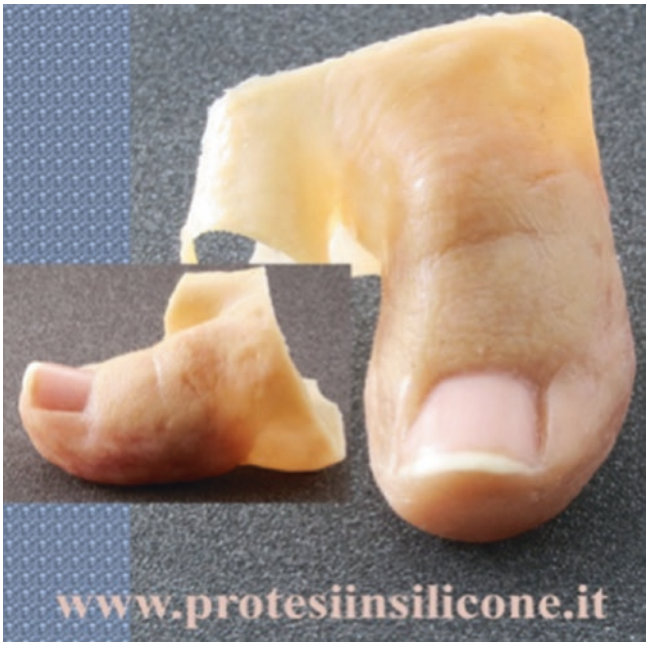

Fig. 27.9 Example of 3D-printing technology used for a silicone prosthesis of hallux (source: http://www.protesiinsilicone.it/wp-content/uploads/2016/02/finger-foot-all.jpg. Author: Erica Buzzi. Permission delivered from Author)

functional, effective, and comfortable, but it may be esthetically unacceptable. Full-length shoes with a rigid rocker sole are thus usually recommended.

Footwear in Charcot's Foot. Charcot's foot is characterized by complications of bones and joints of the foot in patients with diabetes and peripheral neuropathy. However, this is not always the case as the condition may occur in other forms of neuropathy, such as syringomyelia and tabes dorsalis. A clear case of Charcot's foot is characterized by a complete involvement of the bones and joint structures and the loss of the structural organization of the foot. In its most typical form involving the tarsal bones, there is a collapse of the foot arches and of the plantar roof; the foot then changes its proportion, becoming shorter and squat and the plantar surface assuming a rocking profile; as a direct consequence of these dramatic morphologic changes, there is the onset of very high pressures at midfoot, and the area becomes at high risk of ulceration [25] both for the objectively high level of stress and, even worse, because this area is not "designed" to support high loads (as is instead for heel and metatarsal area). Corrective intervention in these patients involves different phase-related options. In the less dramatic case in which the bone involvement is detected before bone collapse, the use of a plaster cast, followed by a corrective strategy involving the use of a plantar support of the arch which enables the stabilization of the lesion, thus hopefully preventing the structural damage of the foot. In other, more dramatic cases, a diagnosis is made when the bone structure has already deteriorated and the tarsal bones have lost their shape. The use of a plaster cast is necessary also in this case at least until the lesion has been stabilized; in some cases, it may take up to 6 months, associated with monitoring of the skin temperature. Subsequent corrective strategies will largely depend on the ensuing structural deformity: if the patient is able to wear shoes, albeit customized footwear, surgical intervention may not be necessary, while surgery is usually indicated otherwise. Corrective strategies aim at reducing plantar high plantar pressures and subsequently the risk of ulceration especially at the midfoot area: as for footwear, rocker sole shoes should be used but the double rocker sole is recommended which, opposite to what all the other rocker soles do, redistribute plantar pressures while offloading the midfoot area. Literature showed that by using proper footwear, even commercially available therapeutic footwear associated with custom foot orthoses, more than 50% of patients with Charcot arthropathy at the midfoot level can be successfully managed without surgery [26].

Aids for Patients with Active Lesions. As we have already highlighted, patients with peripheral neuropathy tend to develop ulcers which are frequently associated with the plantar regions undergoing the highest local compressive load. Often, patients are not able to, and they should not, stay in bed for 4 or 6 weeks, which is ideally the time required to heal an ulcer in patients with normal arterial circulation and an absence of significant complications (e.g., overlapping infections). Also for this reason, often neuropathic ulcers do not heal at all, due to the continued load placed on the ulcer site during walking. It is fundamental in these cases to provide for an adequate unloading in order to support healing [27]. Several options are available to ensure unloading in patients with active ulcers [28]. Most commons among these are: total contact cast; other casts/boots (Air cast, StabilD, Optima Diab, Vacodiaped, walking casts…); and temporary shoes. Total contact cast (TCC) is the most extensively studied technique; it offers total offloading of the ulcer as well as the rapid mobilization of the patient who may resume almost normal activities immediately. As shown in several studies, TCC has become the gold standard for the treatment of diabetic foot ulcers [29, 30]. It allows the immobilization and the complete offloading of the tissues of the ulcers, while redistributing pressures over a wide surface of the foot and along the lower part of the leg. However, the use of TCC must follow specific indications, i.e., neuropathic lesions can be treated when in the total absence of infections, while the TCC solution is definitely contraindicated in the presence of ischemic lesions and/or infected lesions. Furthermore, its use is contraindicated as well in blind patients and in patients with pathological obesity or ataxia [31]. In a TCC-based treatment, the plaster casts cover the lesion and are removed and substituted weekly, in order to ensure an optimal fit as the edema withdraws, and to inspect the wound. Alternatively,

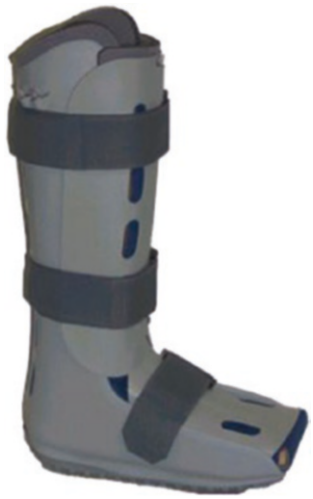

Fig. 27.10 Commercial example of offloading walker by Aircast: control of high pressures at the ulcer site is obtained by means of subpatellar unloading

the scotch cast can be used, which is a sort of easily removable boot made of stiff, light material padded with wadding in order to reduce pressure. This procedure is suitable for elderly patients who do not tolerate the plaster cast, or in those cases where ulcers are situated in difficult areas [28, 32]. Other commercial techniques involve the use of stirrups or other pneumatic means of subpatellar offloading (Aircast walker, Fig. 27.10). One of the limitations to the use of these walkers may be their easy removability, which may allow patients to wear them discontinuously, thus seriously affecting adherence to (and effectiveness of) treatment. On the other hand, this special feature—i.e., removability—also represents their strength, since it renders them usable also in those clinical conditions where the lesion requires strict, almost continuous monitoring. To solve this problem, Armstrong et al. [33] have proposed the use of the "instant" TCC, basically a walker which is rendered non removable for example by wrapping it with cast material. This solution can have all the advantages of the walker without the disadvantages related to a poor adherence. Despite the high cost of these aids has limited their widespread use for years, literature reported about interesting, sometimes controversial findings with respect to the use of these and other offloading alternatives to cope with diabetic ulcer healing. Before 2000s, valuable studies in this field [34, 35] agreed that removable walkers did not seem to be more beneficial than the TCC. However, in 2007 [36] a randomized perspective study by Piaggesi et al. found that the Optima Diab walker (Fig. 27.11) was "as safe and effective as TCC in the management of diabetic foot ulcers, but with lower costs and better applicability." Similarly, in 2010 a RCT by Faglia et al. [37] proved that the Stabil-D cast walker (Fig. 27.12), although removable, was equivalent in efficacy to the TCC in terms of ulcer size reduction and total healing rate, but easier to be used.

Fig. 27.11 Commercial example of offloading walker by Molliter (Optima-Diab)

Fig. 27.12 Commercial examples of offloading walker by Podartis (StabilD)

In 2011, Hunt review [38] confirmed that removable-cast walkers seemed equally effective as TCC, with the added benefit of requiring less technical expertise for fitting; however, to reach this outcome they must be rendered

irremovable. At the same time the review reported that nothing could be said—at the time of its publication—about the possible effectiveness of pressure offloading with felted foam or pressure-relief half-shoe.

The need for rendering removable walker nonremovable is stressed in a 2012 review paper by Bus [39], which supports the concept that "more effective healing of foot ulcers can be obtained when using nonremovable instead of removable offloading treatment."

In a partial disagreement with [36, 37], in 2014 a review by Healy et al. [40] concluded that due to the lack of RCTs it was not possible to make strong conclusions on the examined interventions effectiveness, among which the removable cast walkers. However, they found that the latter resulted to be the most effective among the examined removable devices (removable cast walkers, therapeutic shoes, temporary half, or heel relief shoes).

Enough evidence was instead found in two 2016 reviews by Bus et al. [41] and by Elraiyah et al. [42], respectively. They both confirmed nonremovable offloading was proved to be more effective than removable offloading.

However, quite surprisingly, in 2016 Piaggesi et al. [43] published the results of a randomized prospective trial suggesting that "a walking boot was as effective and safe as TCC in offloading the neuropathic diabetic foot ulcers, irrespective of removability."

Nonetheless the good clinical outcomes in the presence of neuropathic diabetic foot ulcers—and all the above literature only refers to such scenario—, the plaster cast is unsuitable in some conditions. Other aids must be used in these cases, as in example temporary shoes as talus shoes which enable an unloading of the lesion in the forefoot due to the absence of a sole in the front part of the shoe (Fig. 27.13). Using this healing device, the patients walk by loading only the rear foot. This type of footwear is particularly indicated in young persons who do not present problems of equilibrium. Other aids include temporary footwears with extra volume (extra deep 1/2″ or super deep 3/4″) and rigid rocker sole

Fig. 27.13 Talus shoe: unloading of a forefoot ulcer is a consequence of the patient walking by only loading the rearfoot

Fig. 27.14 Temporary footwear with extra volume and rigid rocker sole

(Fig. 27.14). The extra space is necessary to content a bigger foot because of the edema and of the infection that can be present, an insert that can be grossly molded to form a depression in which the ulcerated area can be accommodated and unloaded, and bandages that can be different in volume according to the needs of the ulcer. The rigid sole guarantees the immobilization of the metatarsal–phalangeal joint and a reduced load at the level of metatarsal heads [44]. The foot ulcer unloading given by temporary footwear is not equivalent to that of total contact cast or walking casts [28], and the most recent literature summarized above does confirm this finding; however, this kind of device may have other advantages such as its wider usability because of the absence of adverse effects even in the presence of those complications which prevent the use of more effective solutions, a better acceptance and therefore better compliance by the patients because of their feeling of a quite normal lifestyle with the possibility of having little walks or driving the car even during the healing phase of their foot ulcers.

Relevant Literature Update and Innovation

Relevant issues raised in the most recent literature are here below briefly reported and discussed, which deal with several aspects of the care of the diabetic foot through footwear and plantar orthoses.

Shear Stress [22]. Literature addressed the concept that shear stress, as well as compressive forces, plays a critical role in the development of plantar ulcers and deserves equal attention. Valuable studies showed that elevated pressures, i.e., vertical local compressive forces, are not the only factor to associate with ulcer occurrence [45], since they do increase ulceration risk, but the correlation is low between the maximal pressure sites and the prospective ulcer sites. Lavery et al. encouraged to study shear stress deeply, also considering that during a single stance the same local area under the foot, and in particular the forefoot area, can

experience stresses in opposite directions due to braking forces in the contact phase and propulsive forces in the push-off phase. The main problem with the management of shear stress is that it is hardly measurable, thus an objective evaluation of the effectiveness of the proposed solutions is still far to be reached especially in clinical routine. A 2013 review, in fact [46], reported that meaningful variables of plantar mechanical stress reasonably relate to vertical pressure, shear stress, and temporality of loading, but at the time of the review publication in-shoe peak plantar pressure (PPP) seemed the only reliable variable that can be used to prevent diabetic foot ulcers. Although it is a poor predictor of in-shoe PPP, barefoot PPP seems complementary and may be more suitable when evaluating patients with diabetes mellitus and peripheral neuropathy who seem noncompliant with footwear." Nonetheless, efforts to manage shear stress are recommended [47–49], and some key points are reinforced in a 2015 review [50]. Mainly on the basis of the above papers, the review summarizes the following concepts: diabetic foot ulcers often occur at locations of calluses, the latter originated not only from high plantar pressures but also from frictional shear forces; tissue breakdown occurs more rapidly when shear is increased; the damage done by repetitive friction load does not begin at the outermost layer of the skin, since friction causes shear forces to act between the skin layers; ensuring that the shoe size and shape are appropriate for the foot is perhaps the easiest way to reduce shear inside of a shoe, since a loose shoe and a tight shoe both have the potential to increase shear, friction, and/or pressure on the foot; another way to decrease friction and shear is to "lubricate" the surfaces moving against one another. The last issue, i.e., lubrication of surfaces, may be addressed by using shear-reducing socks with a low coefficient of friction (COF). Traditional cotton socks have a relatively high COF, especially when damp. The use of double socks allows the shear to take place between the layers of socks as opposed to between the skin and sock or sock and insole. Lubrication can also be implemented by using a low-friction interface: a polytetrafluoroethylene material called ShearBan® [51] is widely available to the orthotic, prosthetic, and pedorthic industry. The self-adhesive material can be adhered virtually anywhere inside of a shoe, brace, or prosthetic socket. It is also heat moldable.

Foot Orthoses. Custom therapeutic insoles tailored to contours of the barefoot pressure distribution and shape of a patient's foot can reduce plantar pressures in the metatarsal head region to a greater extent than conventional custom insoles [52]. Main features in the design and construction of foot orthoses for a diabetic neuropathic foot have been helpfully summarized in the 2010 review by Janisse et al. [22]; they are briefly reported and discussed here below, with only minor update by the most recent literature [42, 50, 53]:

- Foot orthoses, in any diabetic neuropathic case, need to be custom-made, a feature that has been found extremely effective in reducing relapse rate [42]. Their main aim is to improve pressure distribution via total contact between orthosis and foot. In general, they should be made of a soft, conformable, cushioned top layer in conjunction with a firmer, supportive base layer (semirigid configuration, more useful than only accommodative [50]). The contours of the plantar surface of the foot should be filled with material and then planned flat on the bottom so that when the patient stands on the orthosis the entire plantar surface of the foot is weight bearing.
- Most used material for top layer is Plastazote, which is a moldable, static dissipative, nitrogen-charged, closed-cell, cross-linked polyethylene foam. Unfortunately, its lifecycle is relatively short; usually, it is supported by a thin layer of polyurethane foam or EVA. The base layer must be supportive and shock absorbing for an insensate foot, and easily adjustable; thus, rigid thermoplastic material or carbon composite should be avoided, while suitable materials are EVA or cork composite with EVA, thermoplastic, latex rubber, or fiberglass. These materials—Shore A in between 50 and 60—are also interesting since they can be used in conjunction with CADCAM systems for the construction of foot orthoses under machine controlled conditions.
- In the preparation of orthoses, semi-weight-bearing mold should be used rather than offloaded or full weight-bearing molds, since it has been showed that this solution delivers products which best address the foot needs during standing and walking.
- Proper pads may be added to the above custom-made orthoses proximal to specific areas which ask for further pressure reduction. In 2008 Actis [54] proposed the use of custom-made insoles with a certain number of plugs of softer material inserted in the forefoot area to further reduce its loading. The study, based on the use of finite element models, dealt with the design of total contact inserts (TCIs) with special "solutions" improving the technique of the added pads, and showed that these last customized inserts with softer plugs distributed throughout the regions of high plantar pressure reduced the peak plantar pressure more than the TCI alone. The strength of this solution is the total lack of edge effects, associated with a greater degree of flexibility for customizing orthotic devices than current practice allows. The study was focused on a Plastazote Shore 35 TCI of 1.27 cm height, heightened to include the medial arch support and used with standard therapeutic shoes (SoleTech shoes style) [55]. Seven Poron plugs—4 mm in diameter—were inserted into the forefoot area of the TCI, spaced 1 mm, and penetrated 7 mm into the TCI. This solution resulted preferable to the single plug design which showed an

undesirable secondary pressure peak 20 mm distally from the center of the metatarsal head at the end of the plug.

A 2011 review [53] also addressed injectable silicone as a possible alternative strategy to reduce plantar pressures and attenuate the risk for ulceration. The review was quite positive with respect to the treatment, stating that: silicone is a biocompatible material that can be safely injected into plantar soft tissue to augment tissue thickness and prevent the development of ulceration; this enhancement to the subcutaneous layer is remarkably well retained and is a generally well-adopted procedure in the clinical setting. Up to now, no further evidence of extensive clinical use of this strategy has been found in the peer-reviewed literature.

As already introduced in previous paragraphs, a relevant innovation is expected in the very next future and is indeed already in progress also in the field of foot orthoses: the 3D modeling and printing. Its worldwide dissemination is driving valuable research towards more and more performing materials and personalized solutions. As an example of excellent work in progress, some departments of the Fraunhofer Institutes (Germany) are currently deeply involved in designing, prototyping, testing and setting up a whole process for 3D printing customized insoles for diabetic patients. An innovative thermoplastic polyurethane, arranged in three-dimensional structures, is currently under study, the final products being likely ready for the market within 2 years (link: http://phys.org/news/2016-11-d-customized-insoles-diabetes-patients.html, check Nov 2016).

Footwear Prescription. A very well done review dealing with the issues of foot treatment and footwear prescription in case of diabetic foot at risk of ulceration was delivered by Bus et al. in 2008 [4]. In the paper, a thorough investigation is conducted to better understand why, despite the wide prescription of custom footwear especially after ulcer healing, the evidence for the effectiveness of this solution to cope with pressure and to prevent ulcer recurrence is still quite poor. As possible factors, Authors indicate: the lack of standardized/systematic approach in footwear prescription and evaluation, and a relevant variability across patients in the offloading effect of different footwear interventions. Also the various combinations of foot and ankle alterations in both structure and function may contribute to such a variability. Most frequent foot abnormalities to deal with when prescribing footwear are callus formation, prominent metatarsal heads, claw/hammer toe deformity, hallux valgus, and limited joint mobility; in some cases, even though the prevalence is low, midfoot deformity is present due to Charcot neuro-osteoarthropathy. Further, foot imaging may also reveal plantar foot muscle atrophy, distal displacement of the protective metatarsal heads fat pads, reduction in the thickness of submetatarsal head fat tissue, increase of subphalangeal fat tissue thickness, plantar fascia thickening. Generally speaking, while eventually dealing with all the above issues, proper footwear prescription should deal with redistribution of mechanical stress not only at the plantar surface but also on the dorsum of the foot. This may involve the fabrication of accommodative insoles that follow the contours of the plantar foot surface (total contact) and also the use of fully customized (therapeutic or orthopedic) shoes with eventual corrective elements, such as arch supports, metatarsal pads and bars, or specific outsole configurations. Keeping ulcers healed thus seems to be a difficult task, and the reported annual ulcer recurrence rate varies between 8 and 59% [56]. Improvement in the outcome of footwear prevention program might come, as suggested in the review, from a more systematic approach to footwear prescription. The first systematic approach—the pyramid approach—was proposed in 2001 by Cavanagh et al. [57]. Basically, the "pyramid" is formed by: (1) patients without foot deformity and a relatively low activity level, who may be recommended to use proper athletic shoes; (2) patients with increasing degrees of foot deformity and activity level, who need more protective, biomechanically effective and eventually more customized solution; and (3) patients with severe deformities and an active life style, who need fully customized solution. Again in 2001, a footwear construction algorithm was delivered by Dahmen et al. [48] which is mainly based on medical condition and type of deformity. Main features of the algorithm are insole design, shoe height, rigidity of the outsole, and pivot point location. The 2008 review however, found no scientific evidence related to the above approaches. As the literature shows [4], current knowledge on the efficacy of footwear design features is mainly based mainly on foot pressure studies. In this sense, in 2009 a proposal was published [58] to objectively quantify the efficacy of a footwear intervention by using a proper indicator; the Authors suggested using a target of 200 kPa of in-shoe peak pressure. In 2013, this Chapter Authors [59] published a similar test protocol for footwear prescriptions, where peak pressure thresholds were modulated according to foot regions and risk level, and gait line pattern was also taken into account as an additional indicator. Bus et al. [60] also indicated the solution of a footwear optimization based on successive footwear assessment and modifications, with the final target to reduce peak pressures below 200 kPa or 25% compared to baseline. These proposals are quite interesting, and the establishment of evidence-based guidelines for proper footwear prescription and evaluation is encouraged [4], since at present the success of most footwear prescriptions is still evaluated clinically on the basis of whether or not the patient develops lesions. However, it is mandatory to keep in mind that, when efficacy has to be proved through an objective measurement procedure, this needs to be standardized and assessed as for accuracy of the measurement instrumentation and correctness, appropriateness, and comparability of the measurement

protocol. In case these essential requirements are not fulfilled, the risk of wrong conclusions and consequent wrong therapeutic intervention indeed becomes very high [61, 62]. Successive valuable papers investigated evidence of footwear prescription effectiveness not only as an isolated treatment, but also in conjunction with education programs as indicated by the International Consensus on Diabetic Foot [63, 64].

Effectiveness of footwear prescription: main criticalities. The most recent reviews on the topic raised the attention on two very critical issues, namely the need for a greater adherence to the treatment and the importance of integrated models of foot care [39, 50, 65–69]. Briefly, the 2012 review by Bus et al. [39] well stressed the following concepts: clinical outcome of a footwear prescription is not only associated with the management of foot plantar pressure but also with non-biomechanical factors including patient behavioral factors—in example the type and intensity of daily physical activity—and, most important, adherence to prescribed treatment; not even the best footwear or offloading device or instruction given (e.g., do not walk barefoot at any time) will be effective, in fact, if it is not used or adhered to; adherence has been found as low as 25–28%; ways to improve adherence could include the prescription of multiple pairs of footwear for use inside and outside the house, the design of more attractive footwear without loss of functionality and better education and communication strategies. With specific focus on reduction of re-ulceration, the 2014 review by Lazaro-Martinez et al. [67] reported the following statements: assessment of biomechanical alterations define a foot type position; examining foot structure and recording plantar pressure could help in appropriate insole and footwear prescription and design, but patient education and compliance should be taken into consideration for better therapy success; diabetic footwear is really effective when it is worn for at least 60% of the time, however all the rates of compliance are lower than this; patient education and awareness should be always part of the treatment. The concept of integrated models of care is well explained in an interesting 2015 review by Janisse [50]. The focus of the review is on the *pedorthic management of the Diabetic foot*: they suggest that pedorthic devices may be successfully integrated into a comprehensive treatment plan for patients with diabetes and foot ulcers. Integrated care is also recommended in the 2016 review by Robinson et al. [66], who state that "given the multifactorial nature of the neuropathic foot, treatments must be multifaceted and patient-specific to effectively address the underlying disease processes. While systemic issues such as peripheral arterial disease are treated by physicians, local issues such as foot deformity are managed by orthotists." Again in 2016, van Netten et al. [65] stated that "to prevent recurrence, some evidence exists for integrated foot care when it includes a combination of professional foot treatment, therapeutic footwear and patient education."

Another criticality still impacting on the effectiveness of footwear treatment is the *Proper Shoe Fitting*. Twenty percent of ulceration in patients with diabetes is a result of ill-fitting footwear [70, 71], partly due to the fact that when purchasing footwear, patients may be strongly influenced by fashion and financial resources. Recent studies proved that, despite a correct shoe fitting is mandatory for the success of a footwear therapeutic intervention in diabetic patients both in primary and secondary prevention, a lot of patients still wear ill-fitting shoes. Furthermore, if neuropathy coexists with peripheral vascular disease, tight shoes may be even more problematic because it may induce lesions on the area with localized high pressure. In a paper by Harrison et al. [72], a study was described which included 100 diabetic patients: 1/3 of them resulted to be wearing the correct shoe on either foot while sitting or standing; however, only 24% of patients were wearing shoes that were of the correct length and width for both feet while sitting and 20% upon standing. In most cases, the ill-fitted shoes were too narrow. The authors suggest that "shoe should be considered of an incorrect length when the difference between foot length and shoe length (in shoe size) is more than half a size difference, and of an incorrect width when the difference between foot and shoe width is greater than one width size." According to the authors, main reasons for selecting wrong shoe size are: (a) adults do not get their feet measured on a regular basis: foot size should always be checked properly prior to shoe purchase; (b) fashion issues may also be a factor; (c) shoe sizes among shoe manufacturers are not standardized; and (d) many manufacturers do not make half sizes and shoes of varying widths: often patients have to buy longer shoes to get the width fitting they require to accommodate their feet. We believe that another relevant reason should be added to the above list, i.e., the underestimation of the volume to be deserved to custom insoles. A paper from Parnes [73] underlines that, besides the already reported drawbacks of ill-fitting shoes, the increased risk of fall should be taken into account, too. Of course, risks of damages from the above "mistakes" can be—and often are—mitigated by a prompt intervention of professionals when testing the footwear prescription before approval and eventual reimbursement. However, if this test procedure is not performed correctly, or if the footwear choice is not examined by a professional as it may happen for very low risk patients, the risk of damage from footwear bad fitting may indeed be very high. In 2015 the relevance of good fitting has been further stressed by the already cited review by Janisse [50], where the role of the pedorthist has been suggested as pivotal in the footwear treatment of the Diabetic Foot.

Hints from updated International Recommendations and Guidelines. This paragraph will briefly report on the most recent dissemination activities of IWGDF (International Working Group on Diabetic Foot) and ADA (American Diabetes Association), the former at the very specific level of Diabetic foot care and the latter at the more general level of the Diabetes whole process of care.

As already mentioned in the previous paragraphs, IWGDF published updated Guidelines in 2015 for Prevention and Management of foot problems in Diabetes [13]. Guidance documents were all based on Consensus Documents, often published by the IWGDF members in the form of systematic reviews. This was the case for the specific issue of "Footwear and Offloading" Guidance document, based on the already cited 2016 review by Bus et al. [41, published online in 2015]. We believe it is helpful repeating here the key points of the evidence-based guideline on the specific aspect of footwear:

- Patients with an at-risk diabetic foot should be urged not to walk barefoot but to wear protective footwear both at home and outside.
- Although no evidence exists, it is often apparent clinically that even extra-depth footwear may not accommodate a foot with significant deformity. In such cases, custom footwear is recommended.
- Therapeutic shoes can be used for preventing plantar ulceration in the at-risk diabetic foot.
- To achieve maximal reduction of peak plantar pressures in footwear prescription, custom-molded insoles should be incorporated in the therapeutic footwear as long as sufficient space exists.

Finally, it is only worth to underline here that, according to the IWGDF practical Guidances, inappropriate footwear do represent an evidence-based risk factor.

The very last dissemination effort by ADA has been published in Supplement 1 of 2016 Diabetes Care [74]. The Standards and all ADA position statements, scientific statements, and consensus reports are available on the Association's Web site at http://professional.diabetes.org/adastatements. At a very high level, but also applicable to foot care, the following recommendations represent the suggested strategies for improving care in diabetes: (1) A patient-centered communication style that incorporates patient preferences, assesses literacy and numeracy, and addresses cultural barriers to care should be used. (2) Treatment decisions should be timely and based on evidence-based guidelines that are tailored to individual patient preferences, prognoses, and comorbidities. (3) Care should be aligned with components of the Chronic Care Model to ensure productive interactions between a prepared proactive practice team and an informed activated patient. (4) When feasible, care systems should support team-based care, community involvement, patient registries, and decision support tools to meet patient needs. Among the others, it is here worth to note that recommendations for physical activity are reported in the document, which surely rely on and interfere with the achievement and maintenance of an adequate, safe and effective foot care and treatment. Specific issues associated with foot care are discussed in Sect. 9 of the Supplement [75], and the following recommendations addressing FOOT CARE are reported:

- Perform a comprehensive foot evaluation each year to identify risk factors for ulcers and amputations.
- Obtain a prior history of ulceration, amputation, Charcot foot, angioplasty or vascular surgery, cigarette smoking, retinopathy, and renal disease and assess current symptoms of neuropathy (pain, burning, numbness) and vascular disease (leg fatigue, claudication).
- The examination should include inspection of the skin, assessment of foot deformities, neurological assessment including 10-g monofilament testing and pinprick or vibration testing or assessment of ankle reflexes, and vascular assessment including pulses in the legs and feet.
- Patients with a history of ulcers or amputations, foot deformities, insensate feet, and peripheral arterial disease are at substantially increased risk for ulcers and amputations and should have their feet examined at every visit.
- Patients with symptoms of claudication or decreased or absent pedal pulses should be referred for ankle-brachial index and for further vascular assessment.
- A multidisciplinary approach is recommended for individuals with foot ulcers and high-risk feet (e.g., dialysis patients and those with Charcot foot, prior ulcers, or amputation).
- Refer patients who smoke or who have histories of prior lower-extremity complications, loss of protective sensation, structural abnormalities, or peripheral arterial disease to foot care specialists for ongoing preventive care and lifelong surveillance.
- Provide general foot self-care education to all patients with diabetes.

With specific reference to Diabetic Foot Treatment, ADA states that people with neuropathy or evidence of increased plantar pressures may be adequately managed with well-fitted walking shoes or athletic shoes that cushion the feet and redistribute pressure. People with bony deformities may need extra-wide or -deep shoes; if deformities, including Charcot foot, cannot be accommodated with commercial therapeutic footwear, they will require custom-molded shoes. Special consideration is deserved to patients with neuropathy and acute onset of a red, hot, swollen foot or ankle,

and Charcot neuroarthropathy. Foot ulcers and wound care may require care by a podiatrist, orthopedic or vascular surgeon, or rehabilitation specialist experienced in the management of individuals with diabetes.

Recently, ADA itself focused on another very important topic related to prevention and care of Diabetes with a position statement on physical activity/exercise and Diabetes [76]. It is clearly stated that "The adoption and maintenance of physical activity are critical foci for blood glucose management and overall health in individuals with Diabetes and pre-Diabetes. However some concerns are raised and some limitations are suggested when patients have peripheral arterial disease, peripheral neuropathy, local foot deformity and foot ulcers/amputations." To address these needs, shoe industry has recently proposed solutions (Fig. 27.15) to allow a "protected physical activity" and therefore to make physical activity available also for diabetic patients with lower limbs complications. More specifically, "protection" refers to the action of the following devices: (1) insoles, to redistribute pressures under the foot avoiding the appearance of peaks of pressures. Selection of materials is crucial and it should include shock absorber inserts; (2) socks, made with soft materials without seams, with additional protections for the toes and the Achilles' tendon region; (3) shoes, with extra volume to include insoles and foot deformities if present; their upper should be seamless and auto-fitting; their sole should have a rocker shape and should be rigid or semirigid to allow the rolling of the foot without undue pressures on the forefoot.

Fig. 27.15 Example of sport shoes by Podartis for protected physical activity

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Correction to: Quality of Health Care

Marcia A. Testa

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The below mentioned reference was missing in the chapter 25.

Edmonds ME, Blundell MP, Morris ME, Thomas EM, Cotton LT, Watkins PJ. Improved survival of the diabetic foot: the role of a specialized foot clinic. Q J Med. 1986;60(232):763–71

Now this has been included in the references list and the citation and the references were renumbered consecutively and also moved reference Lorig (2001) from 74 to 86 in the references list.

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