

Use of Nevelia Dermal-Epidermal Regenerative Template in the Management of Ischemic Diabetic Foot Postsurgical Wounds

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Abstract

The purpose of this cross-sectional study is evaluate the effectiveness of a dermal-epidermal substitute (DES) composed of 3-dimensional porous matrix of type I, purified, stabilized, bovin-origin collagen (Nevelia, SYMATESE, Chaponost, France) without a subsequent skin graft in the treatment ischemic postsurgical diabetic foot ulcers. This study group was composed of a sample of consecutive diabetic patients with critical limb ischemia and postsurgical wounds. All patients received a preset limb salvage protocol including the application of the DES, but none received a skin graft. Patients were closely followed until wound healing or different outcome. The outcome measures were healing, nonhealing, major amputation, and death evaluated at 1 and 2 years of follow-up. Forty-one patients were included. The average postsurgical wound area was 69.6 ± 50 cm². Twenty-one patients (51%) healed; 10 patients (24%) did not heal after 1 year of follow-up; however, all of them achieved a mean ulcer size reduction >50%; 7 patients (17%) were amputees; 3 patients (7.3%) died. In a later follow-up (2 years), wounds in 8 additional patients healed. Successful revascularization was an independent predictor of healing (hazard ratio = 5.1, 95% confidence interval [CI] = 2.5-14.9; $P = .0001$), the postsurgical ulcer size (>50 cm²) was an independent predictor of nonhealing (hazard ratio = 6.2, 95% CI = 2.1-38.4; $P = .0001$) while recurrence of critical limb ischemia was an independent predictor of major amputation (odds ratio = 3.4, 95% CI = 1.1-4.5; $P = .002$). The DES composed of type I bovin-origin collagen is useful in the treatment of large postsurgical diabetic foot ulcers, even when the skin graft is not a suitable therapeutic option.

Keywords

diabetes, diabetic foot ulcers, lower extremity wound, dermal-epidermal substitutes, wound dressing, wound management

Introduction

Recent advances in diabetic foot (DF) care have allowed limb salvage of complex clinical cases, including ischemic gangrene of significant portions of the foot, which even in the recent past would have been treated by major amputations.¹

Mandatory in this conservative approach is the restoration of adequate peripheral perfusion through revascularization and the removal of all necrotic or nonviable tissues.² Minor amputations of toes, rays, or even of significant parts of the foot are often part of the limb salvage protocol to avoid major amputations. After the removal of gangrenous tissues, the consequent extensive tissue loss needs to be adequately managed.

In many cases the spared tissue is not enough to cover the surgical area and to allow primary intention healing; therefore, other solutions need to be considered.

Negative pressure therapy is already a consolidated option and it helps in regrowing dermal tissues, without any effect on epidermis.^{3,4} Surgical procedures may also be considered,

including muscle or muscle-cutaneous flaps, but these are not always suitable.⁵ Additional therapeutic options come from regenerative medicine with the availability of dermal and epidermal substitutes (DESS).⁶ Specific experiences in DF care have been reported.^{7,8}

Dermal substitutes can be applied on large and/or deep skin defects, allowing the coverage of exposed tendons and/or bones and may be the recipient of an autologous skin graft.⁹

According to our experience, it is not always possible to perform an autologous skin graft over a dermal substitute for several reasons¹⁰: some related to organization, such as the availability and cost of surgical facilities; some related

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to the characteristics of the patients, as there is an absence of a donor site for these very fragile DF patients, or there is a difficult area of the foot to cover as in the case of the calcaneus region; and finally some related to the patients consensus due to the development of a new ulcer.

Recent evidence supports the use of 2-layer dermal substitutes as single-step treatment in promoting healing of full-thickness noninfected, nonischemic foot ulcers, with sizes ranging from 2.7 to 9.7 cm², in diabetic patients.^{11,12}

We report the outcomes of consecutive diabetic patients with critical limb ischemia (CLI), treated according to our limb salvage protocol, in which during the reparative time, a bilayer DES was applied to manage a postsurgical extensive tissue loss but without the subsequent skin graft.

Patients and Methods

This cross-sectional study includes a sample of 41 diabetic patients consecutively followed at the Diabetic Foot Unit of University of Rome Tor Vergata because of a CLI and ischemic foot ulcers. All patients, as part of our limb salvage protocol, were treated by DES to improve the wound management of significant tissue loss of the foot.

The patients were recruited in the period from June 1, 2016, to May 31, 2017, with 1 year follow-up for nonhealing patients followed by another 1 year late follow-up for both healed and nonhealed patients.

Patients with unsalvageable foot, inability to walk, reduced expectancy of life, persistent reduced foot perfusion after revascularization (TcPO₂ [transcutaneous pressure of oxygen] < 25 mm Hg) were excluded.

Patients' general health was optimized. A standard electrocardiogram was routinely performed. Ischemic heart disease was considered in case of previous acute coronary syndrome or coronary revascularization, evidence of angina, and significant changes on electrocardiography. In the case of significant coronary heart disease, cardiac revascularization was performed and CLI was treated only after hemodynamic stabilization. Hypertension was considered in case of blood pressure >130/80 mm Hg or current anti-hypertensive therapy, hypercholesterolemia in case of low-density lipoproteins >70 mg/dL or statin therapy. Patients were considered smokers only in case of smoking habit at the time of treatment.¹³

All patients were treated according to our limb salvage protocol described in detail elsewhere. Briefly, all patients with CLI and a foot ulcer or gangrene were revascularized with an endovascular approach, according to Italian Guidelines.¹⁴ The initial vascular assessment was performed by clinical evaluation and TcPO₂. Diagnosis of CLI was defined according to clinical signs (ulceration or gangrene) and TcPO₂ (<30 mm Hg).¹⁵ Advanced vascular assessment was usually performed by Doppler ultrasound and in some cases by magnetic resonance or computed tomography to

detect arterial stenosis and/or obstruction and to allow interventional radiologists to define the road map of treatment. Percutaneous transluminal angioplasty was indicated in the case of significant arterial stenosis (>50%) or complete obstruction. Before the procedure and for 1 month after, all patients were treated with acetylsalicylic acid (100 mg/die) and clopidogrel (75 mg/die). Later, clopidogrel was discontinued. In the case of intolerance to acetylsalicylic acid or clopidogrel, ticlopidine was administered.¹⁴

Aggressive surgery was applied in the case of abscesses, compartment syndrome, extended gangrene, infected tissues, and open fistulas. In the event of extended infection, debridement was performed immediately, even before revascularization, to limit the progression of infection.

According to the Guidance, in the case of infection broad-spectrum antibiotic therapy was immediately set up, followed by specific antibiotic therapy adapted by culture results if required.² Offloading was always prescribed. Adequate postoperative shoes were prescribed during the acute phase according to ulcer localization and the amount of tissue lost.²

The surgical approach was tackled using an extensive and complete removal of necrotic and nonviable tissue with the aim to ensure an adequate wound bed for the reparative phase including the application of a DES.

Foot perfusion restoration after revascularization was evaluated by TcPO₂, and patients were only considered suitable for treatment when TcPO₂ values were >25 mm Hg.²

After hospital discharge, all patients were followed-up on an outpatient basis. None of the aforementioned patients received a skin graft, and all patients were monitored until secondary intention healing with monthly follow-up.

Dermal-Epidermal Substitutes

The bilayer DES used is a 3-dimensional porous matrix of type 1, purified, stabilized, bovin-origin collagen (Nevelia, SYMATESE, Chaponost, France). The collagen matrix is supported by a strong silicon sheet enabling firm coverage of the wound bed.

According to the classification by Davison-Kotler et al,¹⁶ this DES is an acellular material, with epidermal and dermal properties, with both synthetic and natural origins and both permanent and temporary effects.

The natural component is type I collagen, the main protein component of the human body and the major component of the extracellular matrix. It acts as a scaffold to allow cell migration, adhesion, multiplication, differentiation, and the complete integration of the dermal sheet with the wound bed, to support neo tissue 3-dimensional formation, with customized properties to shape tissue reconstruction. Therefore, its effect may be considered permanent.

Silicon layer is a synthetic structure composed of polyester-reinforced silicon sheeting. The silicon sheet is

suitable for suture. It can act as an epidermal layer capable of protecting the wound from infection and it may guarantee the best environment for healing. It has a temporary effect. The manufacturer's recommended use is that it should remain on the covered area for 3 weeks before a skin graft is performed. In this case series, we did not perform the skin graft in all cases, but instead left the silicon sheet for very long periods from 8 to 12 weeks, to act as environmental protection for the new derma and allow spontaneous epidermal regeneration. On removing the silicon sheet, the remaining wound was managed using standard medication with saline wet gauzes, until final secondary intention healing.

Outcomes

The following outcomes were recorded: healing, nonhealing after 1 year of observation, major amputation, and death. The first outcome achieved at follow-up was the only outcome considered, and the time to event was recorded, which referred to the day in which the dermal substitute was applied. Healing was considered in the case of epithelial viable tissue, which is complete covering of all previous open wounds. Nonhealing was defined as an unhealed ulcer after at least 1 year of follow-up with preserved ability to walk, without signs of infection and limb ischemia, neither requiring new revascularization or major amputation. Major amputation was defined as any amputation above the ankle.

A long-term evaluation was performed after 1 year in healed and nonhealed patients.

The quality of the new tissue was evaluated using the Manchester Scar Score.¹⁷

Informed Consent

Each patient provided informed written consent for every surgical procedure.

Statistical Analysis

Statistical analysis was performed by SAS (JMP12; SAS Institute, Cary, NC) for personal computer. Data are expressed as means \pm SEM (standard error of mean). Univariable logistic analysis was performed for all potential predictor variables according to the detected outcome. All predictors identified by univariate analysis were evaluated simultaneously in a multivariable regression. $P < .5$ was considered as statistically significant.

Results

Forty-one ($n = 41$) subjects were included. The included patients were mainly male (35/41, 83.1%), with a mean age of 64.9 ± 14.1 years, diabetes duration of 20.4 ± 13.5

Table 1. Baseline Characteristics of Whole Population.

Variables	Values
Age (years)	64.9 ± 14.1
Sex (male)	83.1%
Diabetes, type 2	95%
Diabetes duration (years)	20.4 ± 13.5
HbA1c ($\mu\text{mol/mol}$)	64 ± 22
Hypertension	72.5%
Dyslipidemia	75%
Ischemic heart disease	45%
Current smoking	7.3%
Dialysis	10%
Ulcer size (cm^2)	71.8 ± 51.8
Infection	100%
Gangrene	75%
Heel ulcers	10%
Aortoiliac arterial disease	0%
Femoral-popliteal arterial disease	78%
Infrapopliteal arterial disease	95.1%
Below-the-ankle arterial disease	33.3%
Baseline TcPO ₂	15 ± 9 mm Hg
Post-revascularization TcPO ₂	44 ± 16 mm Hg
Recurrence of critical limb ischemia	17.5%

Abbreviations: HbA1c, hemoglobin A1C; TcPO₂, transcutaneous pressure of oxygen.

years, and a mean HbA1c of 64 ± 22 $\mu\text{mol/mol}$. Other patient characteristics are reported in Table 1.

The patients had foot lesions, ischemic and infected, belonging to class D3 of the University of Texas classification.¹⁸ According to our limb salvage protocol, all necrotic tissues were removed and the following minor amputations were performed: amputation of toe(s) 11 cases (27%), ray amputation(s) 14 cases (34%), transmetatarsal amputation 7 cases (17%), partial heel removal 5 cases (12%), and extensive debridement of soft tissue related to necrotic fasciitis 4 cases (9%).

The reasons for not having performed the skin graft are the following: timely availability of beds for hospitalization in 7 cases (17%), absence of an available donor site in 7 cases (17%), difficult area of the foot to cover in 15 cases (37%), and the remaining were because of lack of consensus, 12 cases (29%).

The following outcomes were achieved: 21 patients (51%) healed after 6.7 ± 2.3 months; 10 patients (24%) did not heal after a mean follow-up of 12 months, but all showed a mean size reduction $>50\%$; 7 patients (17%) were amputated because a relapse of CLI after 2.1 ± 0.6 months; and 3 patients (7.3%) died after 1.9 ± 0.3 months.

In a later follow-up, after 12 months from the recorded outcomes, 8 patients in the nonhealed group had already reached healing after 6 ± 3 months, the 2 remaining still unhealed. Seven patients (33% of the healed group) had a



Figure 1. Ischemic diabetic foot ulcer on the plantar region of right allux deep to the tendons managed by Nevelia after peripheral revascularization and surgical removal of gangrene.

relapse of the ulcer; however, these ulcers were small (<2 cm^2) and healed quickly (<1 month). No amputations were recorded.

Quality of healed skin, evaluated using the Manchester Scar Scale, based on 5 parameters: color, skin texture, contour, distortion, and texture gave scores between 1 (excellent) and 2 (good) for all parameters (Figures 1 and 2). No lesion became infected before the removal of the silicon sheet.

The average postsurgical wound area was 71.8 ± 51.8 cm^2 . Healed patients showed a mean lesion size of 63.8 ± 14 cm^2 , not statistically different from the nonhealed patient group that had a lesion size of 78.8 ± 12 cm^2 .

At the multivariate analysis of predictive factors found at univariate analysis, the successful revascularization was independent predictor of healing (odds ratio [OR] = 5.1, 95% confidence interval [CI] = 2.5-14.9; $P = .0001$), postsurgical ulcer size (>50 cm^2) was independent predictor of nonhealing (OR = 6.2, 95% CI = 2.1-38.4; $P = .0001$), and recurrence of CLI was an independent predictor of major amputation (OR = 3.4, 95% CI = 1.1-4.5; $P = .002$). No variable resulted in prediction of death (Table 2).

Discussion

According to our experience, the DES composed of type 1 bovin-origin collagen was an option to improve the management of ischemic DF postsurgical wounds with very large tissue loss.

These results suggest some considerations. There has been a significant reduction in major amputations in diabetic patients due to the availability of limb salvage protocols including distal revascularization.¹⁹ However, frequently, minor amputations have to be performed to avoid major amputations. Therefore, it is very common for these ischemic patients to have large postsurgical wounds that need to be managed both in the hospital and in the outpatient setting, because of the very long recovery time. A surgical approach with the aim of reaching primary healing, although desirable, is not always feasible, and the availability of DES has significantly increased the possibility of managing this kind of patient while sparing significant parts of the foot. However, even if the application of DES should be followed by the application of a skin graft, in the current clinical practice this solution is not always feasible.



Figure 2. Ischemic diabetic foot ulcer localized on the left rearfoot deep to the bone managed by Nevelia after peripheral revascularization and surgical removal of the gangrene.

Table 2. Multivariate Analysis of Independent Predictors of Outcomes (Healing, Nonhealing, Major Amputation) Found at Univariate Analysis.

Variables	Healing			Non-Healing			Major Amputation		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Successful revascularization	5.1	2.5-14.9	.0001						
Dialysis				0.8	0.6-1.2	.6			
Heel ulcers				0.9	0.5-1.8	.3			
Ulcer size (>50 cm ²)				6.2	2.1-38.4	.0001			
BTA arterial disease							0.6	0.4-1.3	.5
CLI recurrence							3.4	1.1-4.5	.002

Abbreviations: OR, odds ratio; CI, confidence interval; BTA, below-the-ankle; CLI, critical limb ischemia.

A question arises: If judging from the patient's baseline it is evident that applying a skin graft, as part of the user protocol of the DES, is not possible, should a physician dismiss the use of this device to manage large wounds in ischemic patients?

Recent experiences have shown that DES utilized as therapeutic tools in treating diabetic neuropathic ulcers give

very good results, with ulcers size from 2.7 to 9.7 cm², significantly smaller than the wounds treated in our experience.^{11,12} Our experience has shown that using the DES in very large postsurgical wounds, even in absence of a subsequent skin graft, has given very good healing rates. In addition to its clinical efficacy, its use might have, in theory, economic advantages such as fast hospital discharge, easy

management in an outpatient setting, and elimination of new additional surgical procedures for skin graft.

Concerns may arise from a very long healing time. Healing time is significantly longer than the healing time observed when the skin graft immediately follows the removal of the silicon layer. In this case, according to Clerici et al,⁷ the average healing time was 74.1 ± 28.9 days with a rate of healing of approximately 90%.

However, in their study, only approximately 50% of subjects were ischemic, no patients were on dialysis, no patients had necrotizing fasciitis, and no patients showed heel ulcers. In comparison, our cohort of patients was only composed of ischemic patients with an aggressive pattern of peripheral arterial disease (33% with below-the-ankle arterial disease), 10% were dialyzed, 9% had a necrotizing fasciitis, and 12% had a heel involvement. These conditions, mainly the presence of CLI, the involvement of foot arteries, the concomitant end-stage renal disease on dialysis, the presence of necrotizing fasciitis, and the involvement of the heel could influence the rate of healing and limb salvage as already reported in literature²⁰⁻²³ and justify the longer healing time.

Certainly, the choice to follow the standard protocol and to perform a skin graft after the application of a DES is the usual choice^{24,25}; however, our experience also supports the use of this type of DES and under which conditions a skin graft is not performed.

DFUs can be sources of unstable scars and increase the risk of recurrence.²⁵ Nonetheless, the quality of the new skin, without scars, observed in our patients could significantly reduce the risk of relapse, mainly in the loading areas.

We do not know whether it is possible to extend our results to other dermal-epidermal templates or if they are specifically related to the type 1 bovin-origin collagen we have used in our experience. Furthermore, it could be interesting to know if this kind of DES is only capable of replacing the lost tissue simply acting as a passive tridimensional template or it also has other biological activities capable of stimulating reepithelization. Further studies are required to clarify these points.

In addition, as highlighted in our results, as major amputations were only reported in patients who experienced a relapse of CLI, the possibility of healing is closely related to successful revascularization and restoration of adequate peripheral perfusion as already reported in previous studies.²⁶ Furthermore, our experience has confirmed that healing was related to ulcer size.²⁷ This concept reinforces the need for early referral to specialized DF clinics, mainly in the case of ischemic and infected wound and when the ulcers are still small and can be easily managed.^{20,28,29}

Limitations

This study, although carried out in a tertiary level DF center with a multidisciplinary approach, is a single-center

study. The data reported are based on a series of consecutive cases and there is no case-control factor to reinforce our results. There is no comparison with other dermal substitutes; however, this case series is strictly related to the needs of our daily clinical practice. Studies with the aim to evaluate the economic impact of this therapeutic approach could be useful.

In conclusion, our experience has shown that the application of a DES without subsequent skin graft could ensure favorable outcomes in terms of ulcer management and healing when a specific limb salvage protocol is performed.

Author Contributions

Luigi Uccioli made a substantial contribution to the design of the work, acquisition, analysis, and interpretation of data. Marco Meloni made a substantial contribution to the analysis and interpretation of data. Valentina Izzo made a substantial contribution to the interpretation of data. Laura Giurato made a substantial contribution to the interpretation of data. All authors revised the article critically for important intellectual content.

Declaration of Conflicting Interests

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References

1. Giurato L, Vainieri E, Meloni M, et al. Limb salvage in patients with diabetes is not a temporary solution but a life-changing procedure. *Diabetes Care*. 2015;38:e156-e157. doi:10.2337/dc15-0989
2. Schaper NC, van Netten JJ, Apelqvist J, Bus SA, Hinchliffe RJ, Lipsky BA. IWGDF guidelines on the prevention and management of diabetic foot disease. <https://iwgdfguidelines.org/wp-content/uploads/2019/05/IWGDF-Guidelines-2019.pdf>. Accessed December 6, 2019.
3. Meloni M, Izzo V, Vainieri E, et al. Management of negative pressure wound therapy in the treatment of diabetic foot ulcers. *World J Orthop*. 201;6:387-393. doi:10.5312/wjo.v6.i4.387
4. Dumville JC, Hinchliffe RJ, Cullum N, et al. Negative pressure wound therapy for treating foot wounds in people with diabetes mellitus. *Cochrane Database Syst Rev*. 2013;(10):CD010318. doi:10.1002/14651858
5. Hasenstein TA, Greene T, Van JC, et al. Soft tissue reconstruction with diabetic foot tissue loss. *Clin Podiatr Med Surg*. 2019;36:425-440. doi:10.1016/j.cpm.2019.02.006
6. Lucich EA, Rendon JL, Valerio IL. Advances in addressing full-thickness skin defects: a review of dermal and epidermal

- substitutes. *Regen Med*. 2018;13:443-456. doi:10.2217/rme-2017-0047
7. Clerici G, Caminiti M, Curci V, Quarantiello A, Fagila E. The use of a dermal substitute (integra) to preserve maximal foot length in a diabetic foot wound with bone and tendon exposure following urgent surgical debridement for an acute infection. *Int J Low Extrem Wounds*. 2009;8:209-212. doi:10.1177/1534734609350553
 8. Rezaie F, Momeni-Moghaddam M, Naderi-Meshkin H. Regeneration and repair of skin wounds: various strategies for treatment. *Int J Low Extrem Wounds*. 2019;18:247-261. doi:10.1177/1534734619859214
 9. Hu Z, Zhu J, Cao X, et al. Composite skin grafting with human acellular dermal matrix scaffold for treatment of diabetic foot ulcers: a randomized controlled trial. *J Am Coll Surg*. 2016;222:1171-1179. doi:10.1016/j.jamcollsurg.2016.02.023
 10. Biswas A, Bharara M, Hurst C, Armstrong DG, Rilo H. The micrograft concept for wound healing: strategies and applications. *J Diabetes Sci Technol*. 2010;4:808-819.
 11. Tchero H, Herlin C, Bekara F, Kangambega P, Sergiu F, Teot L. Failure rates of artificial dermis products in treatment of diabetic foot ulcer: a systematic review and network meta-analysis. *Wound Repair Regen*. 2017;25:691-696. doi:10.1111/wrr.12554
 12. Guo X, Mu D, Gao F. Efficacy and safety of acellular dermal matrix in diabetic foot ulcer treatment: a systematic review and meta-analysis. *Int J Surg*. 2017;40:1-7. doi:10.1016/j.ijssu.2017.02.008
 13. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care*. 2014;37(suppl 1):S14-S80.
 14. Aiello A, Anichini R, Brocco E, et al. Treatment of peripheral arterial disease in diabetes: a consensus of the Italian Societies of Diabetes (SID, AMD), Radiology (SIRM) and Vascular Endovascular Surgery (SICVE). *Nutr Metab Cardiovasc Dis*. 2014;24:355-369.
 15. Norgren L, Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG; TASC II Working Group. Inter-society consensus for the management of peripheral arterial disease (TASC II). *J Vasc Surg*. 2007;45(suppl S):S5-S67.
 16. Davison-Kotler E, Sharma V, Kang NV, Garcia-Gareta E. A universal classification system of skin substitutes inspired by factorial design. *Tissue Eng Part B Rev*. 2018;24:279-288. doi:10.1089/ten.TEB.2017.0477
 17. Beausang E, Floyd H, Dunn KW, et al. A new quantitative scale for clinical scar assessment. *Plast Reconstr Surg*. 1998;102:1954-61.
 18. Armstrong DG. The University of Texas diabetic foot classification system. *Ostomy Wound Manage*. 1996;42:60-61.
 19. Carinci F, Benedetti MM, Klazinga NS, Uccioli L. Lower extremity amputation rates in people with diabetes as an indicator of health systems performance. A critical appraisal of the data collection 2000-2011 by the Organization for Economic Cooperation and Development (OECD). *Acta Diabetol*. 2016;53:825-832. doi:10.1007/s00592-016-0879-4
 20. Prompers L, Schaper N, Apelqvist J, et al. Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study. *Diabetologia*. 2008;51:747-755. doi:10.1007/s00125-008-0940-0
 21. Meloni M, Izzo V, Giurato L, Gandini R, Uccioli L. Below-the-ankle arterial disease severely impairs the outcomes of diabetic patients with ischemic foot ulcers. *Diabetes Res Clin Pract*. 2019;152:9-15. doi:10.1016/j.diabres.2019.04.031
 22. Meloni M, Giurato L, Izzo V, et al. Long term outcomes of diabetic haemodialysis patients with critical limb ischemia and foot ulcer. *Diabetes Res Clin Pract*. 2016;116:117-122. doi:10.1016/j.diabres.2016.04.030
 23. Pickwell KM, Siersma VD, Kars M, Holstein PE, Schaper NC; Eurodiale Consortium. Diabetic foot disease: impact of ulcer location on ulcer healing. *Diabetes Metab Res Rev*. 2013;29:377-383. doi:10.1002/dmrr.2400
 24. Yücel A, Senyuva C, Aydin Y, Cinar C, Guzel Z. Soft-tissue reconstruction of sole and heel defects with free tissue transfers. *Ann Plast Surg*. 2000;44:259-269.
 25. Yammine K, Assi C. A meta-analysis of the outcomes of split-thickness skin graft on diabetic leg and foot ulcers. *Int J Low Extrem Wounds*. 2019;18:23-30. doi:10.1177/1534734619832123
 26. Faglia E, Clerici G, Clerissi J, et al. Long-term prognosis of diabetic patients with critical limb ischemia: a population-based cohort study. *Diabetes Care*. 2009;32:822-827. doi:10.2337/dc08-1223 [Erratum in: *Diabetes Care*. 2009;32(7):1355]
 27. Oyibo SO, Jude EB, Tarawneh I, et al. The effects of ulcer size and site, patient's age, sex and type and duration of diabetes on the outcome of diabetic foot ulcers. *Diabet Med*. 2001;18:133-138.
 28. Vas PRJ, Edmonds M, Kavarthapu V, et al. The diabetic foot attack: "Tis Too Late to Retreat!" *Int J Low Extrem Wounds*. 2018;17:7-13. doi:10.1177/1534734618755582
 29. Meloni M, Izzo V, Manu C, et al. Fast-track pathway: an easy-to-use tool to reduce delayed referral and amputations in diabetic patients with foot ulceration. *Diabetic Foot J*. 2019;22:38-47.