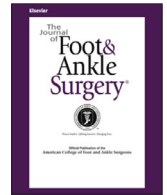




Contents lists available at ScienceDirect

## The Journal of Foot &amp; Ankle Surgery

journal homepage: [www.jfas.org](http://www.jfas.org)

## The Long-Term Outcomes Following the Application of Intralesional Epidermal Growth Factor in Patients With Diabetic Foot Ulcers

Murat Kahraman, MD<sup>1</sup>, Abdulhamit Misir, MD<sup>2</sup>, Turan Bilge Kizkapan, MD<sup>3</sup>, Mustafa Ozcamdalli, MD<sup>4</sup>, Erdal Uzun, MD<sup>5</sup>, Mahmut Mutlu, MD<sup>6</sup>

<sup>1</sup> Surgeon, Department of Orthopaedics and Traumatology, Necip Fazil City Hospital, Kahramanmaraş, Turkey

<sup>2</sup> Surgeon, Department of Orthopaedics and Traumatology, Sanliurfa Training and Research Hospital, Sanliurfa, Turkey

<sup>3</sup> Surgeon, Department of Orthopaedics and Traumatology, Bursa Cekirge State Hospital, Bursa, Turkey

<sup>4</sup> Surgeon, Department of Orthopaedics and Traumatology, Ahi Evran University Faculty of Medicine, Kirsehir, Turkey

<sup>5</sup> Surgeon, Department of Orthopaedics and Traumatology, Ordu University Faculty of Medicine, Ordu, Turkey

<sup>6</sup> Professor, Department of Orthopaedics and Traumatology, Medicana Bahcelievler Hospital, Istanbul, Turkey



## ARTICLE INFO

Level of Clinical Evidence: 4

## Keywords:

adjuvant therapy  
diabetes  
epidermal growth factor  
foot ulcer  
functional outcome  
recurrence

## ABSTRACT

Epidermal growth factor is used as an adjuvant to close the wound in addition to standard care in diabetic foot ulcers. This study aimed to investigate the long-term outcomes after intralesional epidermal growth factor injections in the treatment of diabetic foot ulcers. Thirty-six feet of 34 patients ( $n = 34$ ) with diabetic foot ulcers were included. Patient demographics, Wagner classifications, recurrence and amputation rates, Foot Function Index, Short Form 36, and American Academy of Orthopedic Surgeons Foot and Ankle Module scores were evaluated at the final follow-up examination. The mean age was  $61.000 \pm 13.743$  years. The mean duration of wounds was  $240.200 \pm 146.385$  days. A mean of  $18.125 \pm 4.494$  (range 9 to 24) doses were applied. Wound closure was achieved in 33 of the 36 (91.7%) lesions. A complete response (granulation tissue  $>75\%$  or wound closure) was observed in 29 (87.9%) lesions. The mean time to wound closure was  $52.08 \pm 10.65$  (range 25 to 72) days. At the 5-year follow-up, 4 patients were lost to follow-up because of exitus owing to diabetic complications. Of the remaining 29 patients, 27 were ulcer free. In 2 patients (2 lesions, 6.9%) toe amputation was performed due to ischemic necrosis. The mean Foot Function Index, American Academy of Orthopedic Surgeons Foot and Ankle Core Scale, and AAOS Shoe Comfort Scale scores were  $55.40 \pm 12.15$ ,  $65.92 \pm 17.56$ , and  $56.42 \pm 11.98$ , respectively. Complete wound healing and a low recurrence and amputation rates could be obtained with intralesional epidermal growth factor added to the standard treatment protocol.

© 2018 by the American College of Foot and Ankle Surgeons. All rights reserved.

Diabetic foot ulcer (DFU) is one of the major and devastating complications of diabetes mellitus. It is defined as foot ulceration associated with neuropathy and/or lower extremity peripheral arterial disease in patients with diabetes (1). A full-thickness wound that penetrates the dermis may cause osteomyelitis (2). Approximately 15% of patients with diabetes are expected to develop a lower extremity ulcer. Foot ulcers are the cause of 85% of amputations in patients with diabetes (3). Also, 34% of patients with DFUs develop another ulcer within one year, and the 5-year risk of new ulcer development is approximately 70% (4). Furthermore, the recurrence of ulcers after treatment is one

of the major and challenging problems in the management of DFUs (5).

A multidisciplinary approach is essential in the management of DFUs. Regulation of blood glucose levels, debridement of necrotic and infected tissues, reduction of load and pressure, and revascularization approaches are the mainstay treatment methods when required (6). Additionally, the use of some adjunctive methods such as hyperbaric oxygen therapy and negative pressure wound therapy is accepted (7,8). All of these therapies aim to promote wound healing and decrease amputation rates.

Failure of granulation tissue triggering, histologically abnormal angiogenesis, impaired wound contraction, and aberrant reepithelization are causes of healing failure (9). Epidermal growth factor (EGF) stimulates fibroblast and endothelial cell migration to the ulcer area, the formation of granulation tissue, de novo angiogenesis, wound contraction with myofibroblast activation, and epithelial cell proliferation and migration (10,11). Because of these effects, EGF has begun to be used in the treatment of DFUs.

**Financial Disclosure:** None reported.

**Conflict of Interest:** None reported.

Address correspondence to: Murat Kahraman, MD, Necip Fazil City Hospital, Department of Orthopedics and Traumatology, Erkenez Mh., Recep Tayyip Erdoğan Bulvarı (Gaziantep Kahramanmaraş Yolu) No:14, 46050, Kahramanmaraş/Turkey.

E-mail address: [mkahraman3846@gmail.com](mailto:mkahraman3846@gmail.com) (M. Kahraman).



**Fig. 1.** An application example of epidermal growth factor. First applying dermoepidermal junction all over the wound. Then, deepened and injected homogenously to the entire wound.

Compressive, shear, osmotic and tension forces are effective on cells. Mechanical environment plays an important role on stem cells in both proliferation and differentiation (12). In addition, human mesenchymal stem cells have mechanical memory to maintain their predisposition to certain cell line (13). Mechanical stresses regulate collagen fibril properties, fibrosis, microvascular blood flow, inflammatory response and myofibroblast migration and function (14–16). In DFUs, mechanical stress distribution is impaired (17). Targeting these impaired mechanical forces influences wound healing in DFUs.

The intralesional application of recombinant human EGF is a relatively new method. It has been reported as an effective adjuvant treatment modality for selected patients in the treatment of DFUs in addition to standard care (18). In various studies, EGF was shown to have beneficial effects regarding compete wound healing (6,10,26–28,18–25). However, most of those studies reported the short-term follow-up results. Therefore, this study aimed to present the long-term follow-up results of intralesional EGF injections in patients with DFUs.

#### Patients and Methods

Thirty-six DFUs in 34 patients (28 male and 6 female; mean age  $61.000 \pm 13.743$  years) were treated with intralesional EGF injections in addition to the standard care between June 2012 and February 2013. The standard care in DFUs includes blood glucose

**Table 1**  
Patient characteristics (N = 36 feet in 34 patients)

Characteristic	Means $\pm$ SD or no. (%)
Age (y)	61.00 $\pm$ 13.74
Duration of diabetes (y)	13.96 $\pm$ 9.80
Duration of ulcer (d)	240.200 $\pm$ 146.385
No. of wounds	36 (100)
Lesion size (cm <sup>2</sup> )	22.42 $\pm$ 9.78
WBC (per mm <sup>3</sup> )	8898 $\pm$ 2416
HbA1c (%)	8.357 $\pm$ 4.560
CRP (mg/L)	35.973 $\pm$ 14.090
Amputation (minor)	2 (5.6)
ABI < 0.8	26 (72.2)
ABI > 0.8	8 (22.2)
Sex (patient)	
Female	6 (17.7)
Male	28 (82.4)
Side	
Right	27 (75)
Left	7 (19.4)
Diabetes type (patient)	
1	2 (5.9)
2	32 (94.1)
Etiopathogenic feature	
Ischemic	29 (80.6)
Neurogenic	5 (13.9)
The site of ulcer	
Great toe (plantar)	10 (27.8)
Other toes (plantar and dorsal)	7 (19.4)
Foot dorsum	5 (13.9)
Heel (plantar medial and lateral)	8 (22.2)
Forefoot (plantar)	4 (11.1)
Wagner grade	
2	6 (16.7)
3	28 (77.8)
The most frequent comorbidities (patient)	
Ischemic heart disease	17 (50)
Arterial hypertension	25 (73.5)
Diabetic neuropathy	11 (32.4)
Diabetic nephropathy	6 (17.7)

Abbreviations: ABI, ankle-brachial pressure index; CRP, C-reactive protein; HbA1c, hemoglobin A1c; SD, standard deviation; WBC, white blood cell.

regulation, debridement of necrotic tissues, treatment of infections, correction of deformities, and offloading (6).

The inclusion criteria were patients with type 1 or type 2 diabetes mellitus, patients >40 years of age with a chronic foot ulcer, those with an ulcer area of >3 cm<sup>2</sup>, patients with no or mild foot deformities, and those with Wagner stage 2 and 3 lesions. Patients were excluded if they had a local or systemic infection, pregnancy, malignancy, or previous allergic reactions. Our institutional ethics review board approved this study. Informed consent was obtained from all participants.

The injection was applied as follows: 75  $\mu$ g of EGF vials of 1 mL were diluted with 4 mL of normal saline. Before application, necrotic tissues were sharply debrided, and the wound was washed with normal saline. EGF solution was injected with a 27-gauge  $\times$  0.5-inch insulin needle, first into the dermoepidermal junction all over the wound (Fig. 1), then deepened and injected homogenously to the entire wound (19). Injections were continued 3 times a week for  $\leq$  8 weeks or until a complete granulation response was achieved (Fig. 2).

Systemic antibiotic treatment was used if a wound infection was proven with cultures. Patients underwent serial debridements, 7 days of systemic antibiotic treatment, and continued with oral antibiotics for 21 days. The injections were performed after microbiologic culture confirmation that demonstrated no further infection. All wounds were debrided before the injections. Then, saline and chlorhexidine solutions were used to clean the wound. EGF-interleukin 75  $\mu$ g (Heberprot-P<sup>®</sup> 75, Heber Biotec, Havana, Cuba) was injected intralesionally 3 times per week for  $\leq$  8 weeks. A mean of  $18.125 \pm 4.494$  (range 9 to 24) doses were applied.

The outcomes were investigated after a minimum period of 5 (range 5 to 6) years. Clinicians evaluated patients in the orthopedic surgery, plastic surgery, endocrinology, and infectious diseases clinics before the injections were administered. Thirty-two of the patients were diagnosed with type 2 diabetes mellitus and 2 patients had type 1 diabetes mellitus. Seven patients were treated with oral antidiabetics and 27 were treated with insulin. Of the 34 patients, 21 (61.8%) had a history of surgical intervention for small toe





**Fig. 2.** Complete granulation response in a patient was achieved after 6 injections.

necrosis or recurrent foot infections. The mean duration of wounds was  $240.2 \pm 146.385$  (range 110 to 720 days) days. Right side involvement was seen in 27 patients and left side involvement in 7. In 2 patients, bilateral wound involvement was observed. Wounds were on the great toe in 10 patients, other toes in 7, dorsum of the foot in 5, plantar aspect of the heel in 8, and plantar aspect of the forefoot in 4.

The Short Form 36 (version 1) was used to evaluate the patients' health-related quality of life (29). To evaluate the patients' functional outcomes, we used the American Academy of Orthopedic Surgeons (AAOS) Foot and Ankle Module (with scores ranging from 0 [poor] to 100 [excellent]) and Foot Function Index. The AAOS Foot and Ankle Module includes the Foot and Ankle Core Scale and Shoe Comfort Scale. The Foot and Ankle Core Scale is a 20-item scale to measure pain, function, and stiffness. The Shoe Comfort Scale is a 5-item scale to assess the patient's ability to wear different types of shoes comfortably (30). The Foot Function Index is a region-specific patient-reported outcome score with a 23-item scale. It has 3 subcategories for assessing pain, disability, and activity limitations (31).

The mean, standard deviation, median, lowest, highest, frequency, and ratio were used in the descriptive data statistics. The normality was tested by using the Shapiro-Wilk test. The  $\chi^2$  test was used for comparison of categorical data. Spearman's correlation analysis or Spearman's rho test was used in the correlation analysis. A  $p$  value of  $<.05$  was considered statistically significant. SPSS IBM Statistics, version 22.0 (IBM Inc., Chicago, IL) was used in the statistical analyses.

## Results

Patient demographics, Wagner classifications, recurrence and amputation rates, hemoglobin A1c levels, white blood cell count, and C-reactive protein levels are shown in Table 1. An outgrowth of

a granulation tissue suitable for spontaneous re-epithelization, primary suture or closure with a split-thickness autograft was considered the endpoint.

The mean lesion size was  $22.42 \pm 9.78$  (range 4 to 80)  $\text{cm}^2$ . The lesions were predominately ischemic and Wagner grade 3. Wound closure was achieved in 33 of 36 (91.7%) lesions, with a primary suture in 12 lesions, split-thickness skin graft in 11, and spontaneous re-epithelization in 10 (Fig. 3). In 3 patients, a recurrent wound infection developed and EGF therapy was discontinued. Serial debridements, systemic antibiotic therapy, vacuum-assisted closure, and skin grafting were performed.

Regarding adverse effects, shivering occurred in 11 patients, yawning in 5, pain and burning in 21, and nausea in 17. Cessation of therapy because of these adverse effects was not needed. The occurrence of adverse events increased as the treatment progressed. A complete response (granulation tissue of  $>75\%$  or wound closure) was observed in 29 (87.9%) lesions. Three individuals developed a  $<25\%$  granulation response in the early course of the injection therapy. Serial debridements were added to the treatment between injections, and all 3 wounds closed completely.

There was no significant relationship between the ulcer width and depth and the number of injections ( $p = .192$  and  $p = .528$ , respectively). The mean time to wound closure was  $52.08 \pm 10.65$  (range 25 to 72) days. There was a statistically significant relationship between the



Fig. 3. Spontaneous reepithelization in a patient after 5 injections.

wound width and time to closure ( $p = .013$ ). Also, there was a negative relationship between the Wagner grade and the number of injections ( $p = .041$ ), but not between the Wagner grade, and diabetic foot time ( $p = .192, .528, \text{ and } .855$ , respectively). There was no statistically significant correlation between the hemoglobin A1c levels and Wagner grade ( $p = .225$ ).

At the 5-year follow-up, 4 (11.8%) patients were lost to follow-up because of exitus owing to diabetic heart and cerebrovascular complications. The remaining 27 (79.4%) patients (27 lesions, 93.1%) were ulcer free (Fig. 4). In 2 (6.7%) patients (2 lesions, 6.9%) toe ulcer recurrence was observed, and they underwent toe amputation. The mean Short Form-36 domains, Foot Function Index, AAOS Foot and Ankle Core Scale, and AAOS Shoe Comfort Scale scores at the final follow-up examination are shown in Table 2. Two patients (2 feet) reported minor toe amputations owing to ischemic necrosis without ulceration.

## Discussion

The most important finding of this study is that adding an intralesional EGF injection to the standard therapy is an effective and reliable method for treating DFUs, with low recurrence rates. To the best of our knowledge, this is the first study to report the long-term outcomes after intralesional EGF injections in patients with DFUs.

Multidisciplinary approaches are needed for healing and to prevent amputation in patients with DFUs. Debridement of necrotic tissues and control of infections are required to manage wounds (1). Adding growth factors facilitates the therapeutic effect (32). EGF has been tested and shown to have efficacy in the treatment of DFUs (18,19,33). EGF is a mitogenic and motogenic agent. It stimulates productive cell migration, the formation of granulation tissue, tissue contraction through

myofibroblast activation, and resurfacing through epithelial cell migration and proliferation (10).

The primary goal in the treatment of DFUs is to close the wound completely. EGF is a suitable agent to achieve this goal. Previously, many studies reported successful results, with varying complete wound closure rates and easily manageable adverse events after intralesional EGF injections (10,19,34). Fernández-Montequín et al (33) compared 75 and 25  $\mu\text{g}$  EGF doses and found that the higher dose had a higher and faster complete response than did the lower dose (83% vs 61%). In another double-blind, randomized, multicenter study, the investigators compared 75 and 25  $\mu\text{g}$  doses of EGF with placebo (18). After 8 weeks of treatment, the rates of complete response were 87% with 75  $\mu\text{g}$  of EGF, 73% with 25  $\mu\text{g}$  of EGF, and 58% with the placebo. In another study including 1788 patients with Wagner grades 3 and 4 DFUs, the complete granulation response rate was 75.9%, with 55% complete reepithelization. A 5% person-years relapse rate was reported (35). Valenzuela-Silva et al (36) noted a rate of total wound closure of 58.4% after 75  $\mu\text{g}$  of intralesional EGF was applied. In our study, we also used 75  $\mu\text{g}$  of intralesional EGF (Heberprot-P<sup>®</sup>; 3 applications per week) for 8 weeks. The complete response and complete wound closure rates were 87.9% and 91.7%, respectively. More than 80% of granulation was obtained globally with Heberprot-P<sup>®</sup>, compared with <60% with the standard care alone. This result was compatible with previous studies. Of patients who were treated with 75  $\mu\text{g}$  of Heberprot-P, 77% healed, whereas only 56% healed with placebo injections and standard care (18,33). The continuity of treatment was associated with an improvement in the rates of granulation response and complete wound closure. Since then, local EGF injections have been used for complex diabetic wounds





Fig. 4. Patient before injections and ulcer-free appearance at the last follow-up.

in various clinical trials, demonstrating a favorable risk-benefit balance by enhancing healing and reducing recurrence and the risk of amputation (23,37). Adverse events were mostly mild to moderate (65.6% mild, 28.6% moderate, and only 3.7% severe), with pain and a burning sensation at the administration site as the most frequent adverse event. In our study, the most frequent adverse events were pain, burning, nausea, and yawning. The increased rate of adverse events as the treatment progressed may be associated with increased neovascularization and associated elevated systemic levels of EGF. The treatment was not interrupted because of adverse events.

Gonzalez-Acosta et al (37) reported that intralesional EGF added to standard therapy was associated with a lower rate of major amputation (26.7% vs 8.3%) than standard therapy alone was. In another study, lower major amputation rates (43.1% vs 8.1%) were reported after an intralesional EGF injection was added to the standard therapy (23). In the long term, we observed that 2 of 29 (6.9%) feet underwent toe amputations, without the need for major amputations.

EGF treatment is more expensive than standard therapies. One application cost is 1243.5 USD (In Turkey by the date of May 20, 2018). The cost of 24 applications is 29,844 USD. However, considering the 5-year low recurrence rates that were shown in our study, it may be seen as cost effective regarding total health costs in long-term.

The most common structural foot deformities in DFUs are claw and hammer toes, prominent metatarsal heads, pes cavus, pes equinus, and hallux valgus (38). These deformities change the load distribution on the foot. Foot deformities are one of the major causes of ulceration. The

other major components are peripheral neuropathy, peripheral vascular disease, and minor traumas (39,40).

The best preventive strategy is to decrease the risk factors. In our study, participants had mild foot deformities as claw toe and hallux valgus in 5 toe ulcers, prominent metatarsal heads in 2 plantar forefoot

Table 2

Distribution of SF-36 domains, FFI, and AAOS-FAM scores at the 5-year follow-up examination (n = 30 patients)

Outcome	Mean ± SD
FFI	55.40 ± 12.15
SF-36 v1	
Physical functioning	35
Role limitations owing to physical health	29
Role limitations owing to emotional problems	41
Energy/fatigue	36
Emotional well-being	65
Social functioning	46
Pain	60
General health	31
Health change	38
AAOS-FAM	
AAOS-FACS	65.92 ± 17.56
AAOS-SCS	56.42 ± 11.98

Abbreviations: AAOS-FACS, American Academy of Orthopedic Surgeons Foot and Ankle Core Scale; AOS-FAM, American Academy of Orthopedic Surgeons Foot and Ankle Module; AAOS-SCS, American Academy of Orthopedic Surgeons Shoe Comfort Scale; FFI, Foot Function Index; SF-36: Short Form 36.

Values are mean ± standard deviation or percent.

ulcers, and pes cavus in 3 plantar heel ulcers. Shoe modification was recommended to all patients.

This study has several limitations. First, there is no control group for comparison. Second, we did not use preinjection functional outcome scores. Third, the study was a retrospective case series. Finally, our sample size was relatively small, and it may affect statistical results. The major strength of this study is the first study evaluating long-term outcomes after intralesional EGF application. Further prospective studies with a greater number of participants as well as controls are needed.

In conclusion, our results suggest that the intralesional application of EGF is an effective adjuvant treatment modality. Complete wound healing and low amputation and recurrence rates may be obtained in the long term when EGF is added to the standard treatment protocol.

## References

- Alexiadou K, Doupis J. Management of diabetic foot ulcers. *Diabetes Ther* 2012;3(1):1–15.
- Berlanga-Acosta J, Fernández-Montequín J, Valdés-Pérez C, Savigne-Gutiérrez W, Mendoza-Marí Y, García-Ojalvo A, Falcón-Cama V, García Del Barco-Herrera D, Fernández-Mayola M, Pérez-Saad H, Pimentel-Vázquez E, Urquiza-Rodríguez A, Kulikovskiy M, Guillén-Nieto G. Diabetic foot ulcers and epidermal growth factor: revisiting the local delivery route for a successful outcome. *Biomed Res Int* 2017;2017:2923759.
- Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. *JAMA* 2005;293(2):217–228.
- Frykberg RG, Zgonis T, Armstrong DG, Driver VR, Giurini JM, Kravitz SR, Landsman AS, Lavery LA, Moore JC, Schuberth JM, Wukich DK, Andersen C, Vanore JV. American College of Foot and Ankle Surgeons. Diabetic foot disorders. A clinical practice guideline (2006 revision). *J Foot Ankle Surg*. 2006;45(5 suppl):S1–S66.
- Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med*. 2017;376(24):2367–2375.
- Aktaş S, Baktiroğlu S, Demir L, Kılıçoğlu Ö, Topalan M, Güven E, Mirasoğlu B, Yanar F. Intralesional application of epidermal growth factor in limb-threatening ischemic diabetic foot ulcers. *Acta Orthop Traumatol Turc* 2016;50(3):277–283.
- Khamaisi M, Balanson S. Dysregulation of wound healing mechanisms in diabetes and the importance of negative pressure wound therapy (NPWT). *Diabetes Metab Res Rev* 2017;33(7).
- Stoekenbroek RM, Santema TB, Legemate DA, Ubbink DT, van den Brink A, Koelmay MJ. Hyperbaric oxygen for the treatment of diabetic foot ulcers: a systematic review. *Eur J Vasc Endovasc Surg* 2014;47(6):647–655.
- Berlanga-Acosta J, Schultz GS, López-Mola E, Guillen-Nieto G, García-Siverio M, Herrera-Martínez L. Glucose toxic effects on granulation tissue productive cells: The diabetics' impaired healing. *Biomed Res Int* 2013;2013:256043.
- Berlanga-Acosta J. Diabetic lower extremity wounds: the rationale for growth factors-based infiltration treatment. *Int Wound J* 2011;8(6):612–620.
- Gibbs S, Silva Pinto A, Murlí S, Huber M, Hoh D, Ponc M. Epidermal growth factor and keratinocyte growth factor differentially regulate epidermal migration, growth, and differentiation. *Wound Repair Regen*. 2000;8(3):192–203.
- Barnes LA, Marshall CD, Leavitt T, Hu MS, Moore AL, Gonzales JG, Longaker MT, Gurtner GC. Mechanical forces in cutaneous wound healing: emerging therapies to minimize scar formation. *Adv Wound Care*. 2017;7(2). wound.2016.0709.
- Yang C, Tibbitt MW, Basta L, Anseth KS. Mechanical memory and dosing influence stem cell fate. *Nat Mater* 2014;13(6):645–652.
- Gurtner GC, Dauskardt RH, Wong VW, Bhatt KA, Wu K, Vial IN, Padois K, Korman JM, Longaker MT. Improving cutaneous scar formation by controlling the mechanical environment: large animal and phase I studies. *Ann Surg* 2011;254(2):217–225.
- Wray RC. Force required for wound closure and scar appearance. *Plast Reconstr Surg* 1983;72(3):380–382.
- Malmsjö M, Ingemansson R, Martin R, Huddleston E. Wound edge microvascular blood flow: effects of negative pressure wound therapy using gauze or polyurethane foam. *Ann Plast Surg* 2009;63(6):676–681.
- van Deursen R. Mechanical loading and off-loading of the plantar surface of the diabetic foot. *Clin Infect Dis* 2004;39(suppl 2):S87–S91.
- Fernández-Montequín JI, Betancourt BY, Leyva-Gonzalez G, Mola EL, Galán-Naranjo K, Ramírez-Navas M, Bermúdez-Rojas S, Rosales F, García-Iglesias E, Berlanga-Acosta J, Silva-Rodríguez R, García-Siverio M, Martínez LH. Intralesional administration of epidermal growth factor-based formulation (Heberprot-P) in chronic diabetic foot ulcer: treatment up to complete wound closure. *Int Wound J* 2009;6(1):67–72.
- Acosta JB, Savigne W, Valdez C, Franco N, Alba JS, del Rio A, López-Saura P, Guillén G, Lopez E, Herrera L, Fernández-Montequín J. Epidermal growth factor intralesional infiltrations can prevent amputation in patients with advanced diabetic foot wounds. *Int Wound J* 2006;3(3).
- Dumantepe M, Fazliogullari O, Seren M, Uyar I, Basar F. Efficacy of intralesional recombinant human epidermal growth factor in chronic diabetic foot ulcers. *Growth Factors* 2015;33(2):128–132.
- Ertugrul BM, Buke C, Ersoy OS, Ay B, Demirez DS, Savk O. Intralesional epidermal growth factor for diabetic foot wounds: the first cases in Turkey. *Diabet Foot Ankle* 2015;6:28419.
- Ertugrul BM, Lipsky BA, Guvenc U. Turkish Intralesional Epidermal Growth Factor Study Group for Diabetic Foot Wounds. An assessment of intralesional epidermal growth factor for treating diabetic foot wounds: the first experiences in Turkey. *J Am Podiatr Med Assoc*. 2017;107(1):17–29.
- García Herrera AL, Rodríguez Fernández R, Ruiz VM, et al. Reduction in the amputation rate with Heberprot-P in the local treatment of diabetic foot. *Rev Esp Invest Quir*. 2011;(14):21–26.
- Hong JP, Jung HD, Kim YW. Recombinant human epidermal growth factor (EGF) to enhance healing for diabetic foot ulcers. *Ann Plast Surg*. 2006;56(4):394–398.
- Isikgoz-Tasbakan M, Yildirim-Simsir I, Mermer S, Uysal S, Ozturk M CS. Intralesional epidermal growth factor therapy for diabetic foot ulcers: an evaluation of 15 cases. *Turk J Med Sci* 2017;47(5):1500–1504.
- Singla S, Garg R, Kumar A, Gill C. Efficacy of topical application of beta urogastrone (recombinant human epidermal growth factor) in Wagner's Grade 1 and 2 diabetic foot ulcers: Comparative analysis of 50 patients. *J Nat Sci Biol Med*. 2014;5(2):273–277.
- Tiaka EK, Papanas N, Manolakis AC GG. Epidermal growth factor in the treatment of diabetic foot ulcers: an update. *Perspect Vasc Surg Endovasc Ther* 2012;24(1):37–44.
- Tuyet HL, Nguyen-Quynh TT, Vo Hoang Minh H, Vo Hoang Minh H, Thi Bich DN, Do Dinh T, Le Tan D, Van HL, Le Huy T, Doan Huu H, Tran Trong TN. The efficacy and safety of epidermal growth factor in treatment of diabetic foot ulcers: the preliminary results. *Int Wound J* 2009;6(2):159–166.
- Laucis NC, Hays RD, Bhattacharyya T. Scoring the SF-36 in orthopaedics: a brief guide. *J Bone Jt Surg Am* 2014;97(19):1628–1634.
- Riskowski JL, Hagedorn TJ, Hannan MT. Measures of foot function, foot health, and foot pain: American Academy of Orthopedic Surgeons Lower Limb Outcomes Assessment: Foot and Ankle Module (AAOS-FAM), Bristol Foot Score (BFS), Revised Foot Function Index (FFI-R), Foot Health Status Questionnaire. *Arthritis Care Res* 2011;63(Suppl 11).
- Budiman-Mak E, Conrad KJ, Roach KE. The foot function index: a measure of foot pain and disability. *J Clin Epidemiol* 1991;44(6):561–570.
- Amin N, Doupis J. Diabetic foot disease: from the evaluation of the "foot at risk" to the novel diabetic ulcer treatment modalities. *World J Diabetes*. 2016;7(7):153.
- Fernández-Montequín JI, Infante-Cristiá E, Valenzuela-Silva C, Franco-Pérez N, Savigne-Gutiérrez W, Artaza-Sanz H, Morejón-Vega L, González-Benavides C, Eliseo-Musenden O, García-Iglesias E, Berlanga-Acosta J, Silva-Rodríguez R, Betancourt BY, López-Saura PA. Cuban Citoprot-P Study Group. Intralesional injections of Citoprot-P (recombinant human epidermal growth factor) in advanced diabetic foot ulcers with risk of amputation. *Int Wound J* 2007;4(4):333–343.
- Berlanga-Acosta J, Gavilondo-Cowley J, Lopez-Saura P, González-López T, Castro-Santana MD, López-Mola E, Guillén-Nieto G, Herrera-Martínez L. Epidermal growth factor in clinical practice: a review of its biological actions, clinical indications and safety implications. *Int Wound J*. 2009;6(5):331–346.
- Yera-Alos IB, Alonso-Carbonell L, Valenzuela-Silva CM, Tuero-Iglesias AD, Moreira-Martínez M, Marrero-Rodríguez I, López-Mola E, López-Saura PA. Active post-marketing surveillance of the intralesional administration of human recombinant epidermal growth factor in diabetic foot ulcers. *BMC Pharmacol Toxicol*. 2013;14:44.
- Valenzuela-Silva CM, Tuero-Iglesias AD, García-Iglesias E, González-Díaz O, Del Río-Martín A, Yera Alos IB, Fernández-Montequín JI, López-Saura PA. Granulation response and partial wound closure predict healing in clinical trials on advanced diabetes foot ulcers treated with recombinant human epidermal growth factor. *Diabetes Care*. 2013;36(2):210–215.
- Gonzalez-Acosta S, Calana-Gonzalez-Posada B, Marrero-Rodríguez ILFR. Clinical evolution of diabetic foot treatment with Heberprot-P or with the conventional method. *Rev Cuba Angiol Cir Vasc*. 2011;11(2):11.
- Allan J, Munro W, Figgins E. Foot deformities within the diabetic foot and their influence on biomechanics: a review of the literature. *Prosthet Orthot Int* 2016;40(2):182–192.
- Reiber GE, Vileikyte L, Boyko EJ, del Aguila M, Smith DG, Lavery LA, Boulton AJ. Causal pathways for incident lower - extremity ulcers in patients with diabetes from two settings. *Diabetes Care* 1999;22(1):157–162.
- Wu SC, Driver VR, Wrobel JS, Armstrong DG. Foot ulcers in the diabetic patient, prevention and treatment. *Vasc Health Risk Manag* 2007;3(1):65–76.