ORIGINAL ARTICLES

An Assessment of Intralesional Epidermal Growth Factor for Treating Diabetic Foot Wounds

The First Experiences in Turkey

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Background: Intralesional epidermal growth factor (EGF) has been available as a medication in Turkey since 2012. We present the results of our experience using intralesional EGF in Turkey for patients with diabetic foot wounds.

Methods: A total of 174 patients from 25 Turkish medical centers were evaluated for this retrospective study. We recorded the data on enrolled individuals on custom-designed patient follow-up forms. Patients received intralesional injections of 75 μg of EGF three times per week and were monitored daily for adverse reactions to treatment. Patients were followed up for varying periods after termination of EGF treatments.

Results: Median treatment duration was 4 weeks, and median frequency of EGF administration was 12 doses. Complete response (granulation tissue >75% or wound closure) was observed in 116 patients (66.7%). Wounds closed with only EGF administration in 81 patients (46.6%) and in conjunction with various surgical interventions after EGF administration in 65 patients (37.3%). Overall, 146 of the wounds (83.9%) were closed at the end of therapy. Five patients (2.9%) required major amputation. Adverse effects were reported in 97 patients (55.7%).

Conclusions: In patients with diabetic foot ulcer who received standard care, additional intralesional EGF application after infection control provided high healing rates with low amputation rates. (J Am Podiatr Med Assoc 107(1): 000-000, 2017)

Among persons with diabetes mellitus, the lifetime risk of developing a foot ulcer is estimated to be 15% to 25%. Foot ulcers cause substantial morbidity and impaired quality of life, result in high treatment costs, and are the most important risk factor for lower-extremity amputation.[1] In fact, largely because of these foot lesions, every 30 seconds a person somewhere in the world undergoes a lower-limb amputation as a consequence of diabetes.[2,3] The 5-year mortality in patients with diabetes and critical limb ischemia is 30%, and approximately 50% of patients with diabetic foot infections who have a foot amputation die within 5 years.[4-6] This mortality rate is similar to that of some of the most deadly cancers.[7] The high and growing rates of diabetes in both highly developed and low-income countries throughout the world make managing these foot ulcers a critical public health problem. Although diabetic foot ulcers are mostly attributed to neuropathy, they may develop as a result of vascular failure alone or both. In patients with plantar ulcers caused by neuropathy alone, the use of total-contact casts has the fastest growing and highest rates of healing reported in the literature.[8]

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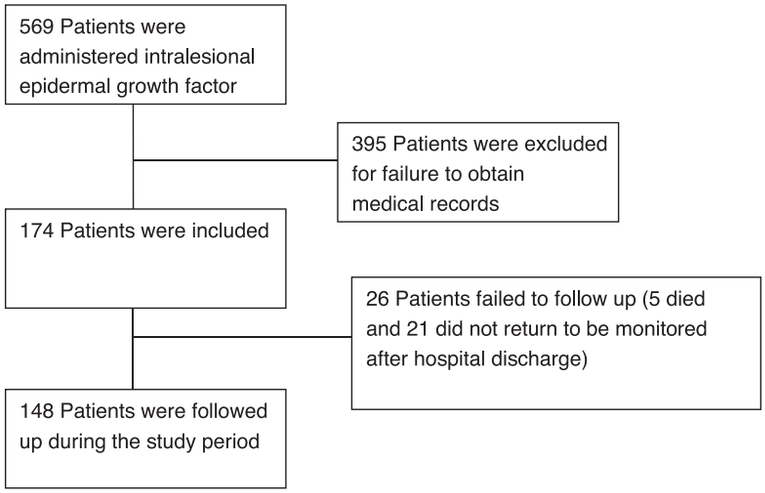

Figure 1. Patient flowchart.

In advanced foot ulcers with neuropathy, vascular failure, or infection, the rate of limb loss remains high despite using the currently available standard therapies (eg, debridement, antimicrobial drugs, wound-healing agents, and hyperbaric oxygen). Thus, new products are urgently needed for the treatment of diabetic foot ulcers.

Epidermal growth factor (EGF) is a 53–amino acid polypeptide isolated from adult mouse submaxillary glands that exerts potent mitogenic activity through binding to a specific cell membrane receptor.[9,10] Epidermal growth factor has both mitogenic and motogenic roles and cytoprotective actions in wound healing. It stimulates 1) migration of fibroblast and endothelial cells to the ulcer area; 2) formation of granulation tissue, including extracellular matrix accumulation, maturation, and de novo angiogenesis; 3) wound contraction by myofibroblast activation and proliferation; and 4) resurfacing of damaged areas by epithelial cell migration and proliferation.[11] Epidermal growth factor plays a dominant early role in wound healing by stimulating keratinocyte proliferation and migration.[12]

Recombinant EGF was first produced at the Center for Genetic Engineering (Havana, Cuba) in 1988. In 2006 it was licensed in Cuba as an adjunct to standard treatment procedures to accelerate wound healing for patients with diabetic foot wounds, whether infected or not. Intralesional EGF has been available as a medication in Turkey since 2012. We herein present what we believe are the first reported results of using intralesional EGF for patients with diabetic foot wounds in Turkey.

Materials and Methods

We conducted a retrospective review of 174 patients from 25 Turkish medical centers treated with intralesional EGF between January 1, 2012, and December 31, 2013. The study was approved by the Medical Ethics Committee of Adnan Menderes University School of Medicine (Aydin, Turkey). Although EGF was administered to a wide range of patients, we included only patients for whom there were complete medical records (Fig. 1). All of the patients had type 1 or 2 diabetes mellitus and foot ulceration and were screened for risk factors known to be associated with lower-extremity complications, eg, advanced age, male sex, long duration of diabetes, previous hospitalization, previous lower-extremity amputation, previous foot infection (especially osteomyelitis), presence of peripheral neuropathy or peripheral vascular disease, greater wound depth, and midfoot or hindfoot ulcer localization. We recorded the data on enrolled individuals on custom-designed patient follow-up forms. The presence of foot abnormalities was assessed by a trained physician according to the methods and recommendations of the International Working Group on the Diabetic Foot (PEDIS [perfusion, extent, depth, infection, and sensation] classification) (Table 1).[13,14]

All of the patients began their EGF treatment as hospital inpatients. The application site was first cleansed and then debrided of necrotic or infected soft tissue and infected bone (in those with osteomyelitis). Several off-loading techniques suggested to the patients included bed rest, crutches, canes, wheelchairs, and walkers. Then patients

Table 1. The International Working Group on the Diabetic Foot PEDIS Classification System

Grade	Perfusion	Extent	Depth	Infection	Sensation
1	No PAD	Skin intact	Skin intact	None	No loss
2	PAD, no CLI	<1 cm^2	Superficial	Surface	Loss
3	CLI	1–3 cm^2	Fascia, muscle, tendon, bone, or joint	Abscess, fasciitis, septic arthritis, osteomyelitis	
4		>3 cm^2		Infection and SIRS	

Abbreviations: CLI, critical limb ischemia; PAD, peripheral arterial disease; PEDIS, perfusion, extent, depth, infection, and sensation; SIRS, systemic inflammatory response syndrome.

received intralesional (into the ulcer base) injections of 75 μg of EGF three times per week on alternate days (generally on Monday, Wednesday, and Friday). Intralesional EGF treatment was initiated only after any infection of the wound was stabilized by surgical debridement and antibiotic drug therapy. Patients with an infected foot wound received both systemic antibiotic (but not topical antimicrobial) drug therapy and EGF administrations during the treatment period. Patients were discharged from the hospital when they achieved clinical stability, and their intralesional EGF treatments were continued in the outpatient setting. Vials of EGF were provided to the medical center as lyophilized powder containing 75 μg of EGF and were stored at 4°C to 8°C. The EGF was dissolved with 5 mL of sterile water for injection; this volume was then distributed throughout the lesion in 0.5- to 1-mL injections starting in the deeper zones.

We had two main outcomes of interest in this study. The first evaluation was based on the percentage of healthy granulation tissue in the base of the ulcer, classified by the method described in previous clinical studies performed with EGF.[15,16] These criteria classify the percentage of granulation tissue as 25% or less (no response), 26% to 50% (minimal response), 51% to 75% (partial response), and greater than 75% or wound closure (complete response). For the second evaluation, we assessed the treatment results not only with EGF but also with various surgical procedures. In this evaluation, closure of the wound was defined as a treatment success.

Patients were monitored daily for adverse reactions to their treatment and were followed up for varying periods after termination of their EGF treatments.

The Kolmogorov-Smirnov test was used to determine the normal distribution of continuous variables. Nonparametric Mann-Whitney U tests were performed for variables without a normal distribution. In addition, we performed receiver operating characteristic curve analysis to determine whether there were cutoff points for statistically significant continuous variables. These variables were divided into two groups according to the cutoff points, which resulted in defining new categorical variables. Proportional comparisons for categorical variables were performed using the χ^2 test. Factors affecting complete response (granulation tissue $>75\%$ or wound closure) and wound closure with EGF administration only were each evaluated by univariate and multivariate logistic regression analyses. Statistical significance was set as $P < .05$.

Results

We found 174 patients who were evaluable for this study, most of whom had type 2 diabetes and were receiving insulin therapy. Most patients were late middle-aged men who had their foot ulcer for approximately 3 months. The demographic characteristics of the patients are shown in Table 2. Patients were hospitalized in various medical centers: 102 (58.6%) in university hospitals, 39 (22.4%) in private hospitals, 20 (11.5%) in public hospitals, and 13 (7.5%) in medical education and research hospitals of the Turkish Ministry of Health. The median intralesional EGF treatment duration was 4 weeks, and the median frequency of EGF administration was 12 doses.

Complete response (ie, granulation tissue $>75\%$ or wound closure) was observed in 116 patients (66.7%) (Table 3). The median time to wound closure in these patients was 40 days. The number of patients whose wounds closed with only EGF administration was 81 (46.6%), and the number of patients whose wound closure occurred in conjunction with various surgical interventions (simple surgical sutures, skin grafts, or free flap) after EGF administration was 65 (37.3%). Overall, wounds were closed at the end of therapy in 146 of the evaluable patients (83.9%) (Table 3). Views of

Table 2. Demographic and Clinical Characteristics of the Study Participants

Characteristic	Patients (Total No.)	Value
Age (mean ± SD [years])	174	61.59 ± 12.8
Male sex (No. [%])	174	126 (72.4)
Duration of diabetes (median [25%–75%] [years])	174	15 (10–20)
Type of diabetes (No. [%])		
Type 1	174	13 (7.5)
Type 2	174	161 (92.5)
Hypertension (No. [%])	166	98 (59.0)
Anemia (No. [%])	157	60 (38.2)
Cardiac failure (No. [%])	157	33 (21.0)
Renal failure (No. [%])	168	44 (26.2)
Receiving renal dialysis (No. [%])	168	33 (19.6)
Smoking (active or history) (No. [%])	153	58 (37.9)
Hemoglobin A_{1c} (median [25%–75%])	174	8 (6–8.75)
Previous hospitalization history (No. [%])	157	114 (72.6)
Duration of diabetic foot ulcer (median [25%–75%] [d])	174	90 (40–240)
Previous foot ulcer at any site (No. [%])	164	95 (57.9)
Previous foot osteomyelitis at any site (No. [%])	154	47 (30.5)
Previous debridement (soft tissue) (No. [%])	165	82 (49.7)
Previous lower-extremity amputation (ipsilateral or contralateral) (No. [%])	174	59 (33.9)
Previous vascular surgery (No. [%])	156	35 (22.4)
Peripheral vascular disease (No. [%])		
Grade 1 (no peripheral vascular disease)	174	79 (45.4)
Grade 2 (peripheral vascular disease, but no critical limb ischemia)	174	62 (35.6)
Grade 3 (critical limb ischemia)	174	33 (19)
Wound depth (No. [%])		
Grade 1 (skin intact)	174	45 (25.8)
Grade 2 (superficial)	174	80 (46)
Grade 3 (fascia, muscle, tendon, bone, or joint)	174	49 (28.2)
Neuropathy (No. [%])	174	124 (71.3)
Ulcer localizations (No. [%])		
Great toe	174	29 (16.7)
Other toes	174	15 (8.6)
Metatarsal	174	18 (10.3)
Dorsal foot	174	34 (19.6)
Plantar foot	174	23 (13.2)
Heel	174	43 (24.7)
≥2 regions	174	12 (6.9)
Patients with infection (No. [%])	174	134 (77)
Infection (International Working Group on the Diabetic Foot classification) (No. [%])		
Grade 1 (none)	174	40 (23)
Grade 2 (surface)	174	34 (19.5)
Grade 3 (abscess, fasciitis, septic arthritis, osteomyelitis)	174	78 (44.8)
Grade 4 (infection and systemic inflammatory response syndrome)	174	22 (12.6)
Osteomyelitis (No. [%])	174	57 (32.8)
Wound size (median [25%–75%] [cm^2])	174	15 (6–30)
Leukocyte count (median [25%–75%] [/mm^3])	108	9,000 (7,000–13,000)
Erythrocyte sedimentation rate (median [25%–75%])	85	51 (25–84)
Hyperbaric oxygen therapy (No. [%])	163	44 (27)
Negative pressure wound therapy (No. [%])	161	49 (30.4)

Table 3. Outcomes After Intralesional EGF Administration

Outcome	Patients (No. [%])				
	No Response	Minimal Response	Partial Response	Complete Response	Total
Wound closure with only EGF administration	0	1	10	70	81 (46.6)
Wound closure by simple surgical suture with EGF administration	0	2	9	10	21 (12.1)
Wound closure by skin graft with EGF administration	1	1	8	32	42 (24.1)
Wound closure by reconstruction processes with EGF administration	0	0	1	1	2 (1.1)
Wound size decreased (but without closure) with only EGF administration	3	5	3	0	11 (6.3)
Recurrent infection	3	3	2	0	8 (4.6)
Major amputation	2	1	0	2	5 (2.9)
Death	2	0	1	1	4 (2.3)
Total	11 (6.3)	13 (7.5)	34 (19.5)	116 (66.7)	174 (100)

Note: See the Materials and Methods section for definitions of the response categories.
Abbreviation: EGF, epidermal growth factor.

the foot taken from four study patients at different treatment stages are shown in Figures 2 to 5.

Of 44 patients with renal failure, 33 were receiving renal dialysis. Complete response (granulation tissue >75% or wound closure) was observed in 28 patients with renal failure (64%) (Table 4). Fifteen of these patients' wounds closed with only EGF administration (34%), and wound closure occurred in conjunction with various simple surgical interventions after EGF administration in 19 patients (43%). Overall, wounds were closed at the end of therapy in 34 of the evaluable patients (77%) (Table 4).

By univariate analysis, factors found to have a statistically significant negative effect on complete response were history of osteomyelitis and, by receiver operating characteristic curve analysis, duration of diabetic foot ulcer of 75 days or more. By logistic regression analysis, history of osteomyelitis, as a categorical variable ($P = .026$), and duration of diabetic foot ulcer of 75 days or longer, as a continuous variable ($P = .003$), had a

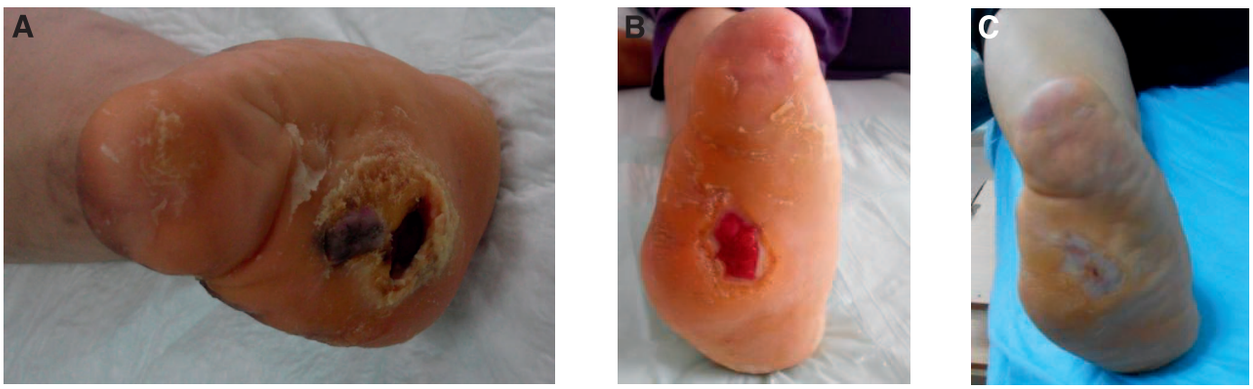

Figure 2. Clinical views of a diabetic foot ulcer before treatment (A), after the sixth intralesional epidermal growth factor application (B), and at the end of treatment (C).

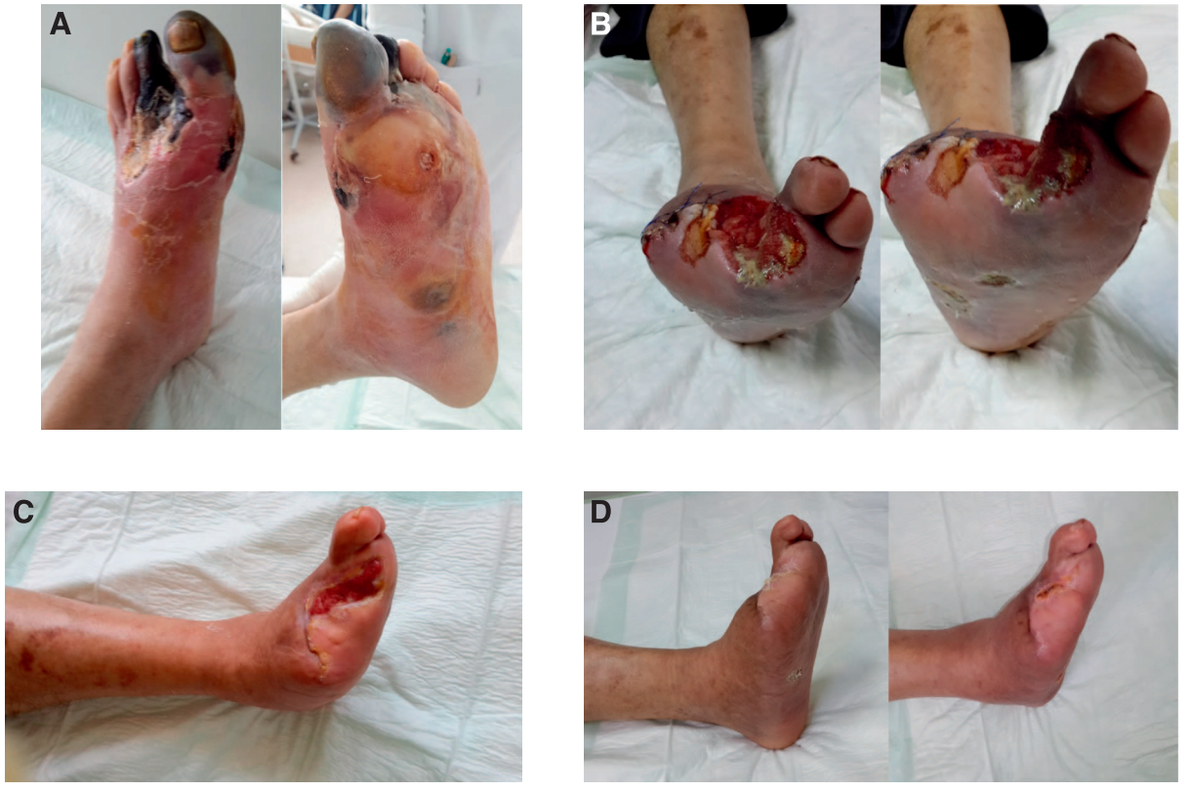

Figure 3. Clinical views of a diabetic foot ulcer before treatment (A), after surgical debridement and minor amputation (B), after the 15th intralesional epidermal growth factor application (C), and at the end of treatment (D).

statistically significant negative effect on the complete response (Table 5). None of the other variables investigated (age, type of diabetes, renal failure, receiving renal dialysis, location and size of the wound, peripheral arterial disease, severity of infection, etc) had a statistically significant association with the occurrence of a complete response.

Table 6 shows a univariate analysis of factors potentially associated with wound closure in patients treated with EGF administration only. By logistic regression analysis, the only two statistically significant factors as categorical variables were male sex ($P = .012$) and history of osteomyelitis ($P = .02$). By receiver operating characteristic curve analysis, patient age of 56 years and older ($P = .003$) as a continuous variable was found to also have a statistically significant negative effect on wound closure (Table 5).

At the end of treatment, five patients (2.9%) required a major amputation. A total of 148 patients were followed up during the study period (Fig. 1) for a median (25%–75%) duration of 6 months (3–9 months). An ulcer recurred at the treatment site in 12 patients (8.1%) during follow-up.

Adverse effects after EGF applications were reported in 97 patients (55.7%) (Table 7). The most common adverse effects were chills and shivering (37.9%) and nausea (22.9%). The most serious adverse effects, ie, infection at the application site (4.6%), syncope (1.7%), and respiratory distress (0.6%), were infrequent. In four patients (2.3%), the EGF dose was reduced because of an adverse effect. In 19 patients (10.9%), the full course of EGF therapy could not be completed as previously planned; in 13 of these patients (7.5%), EGF therapy was discontinued on their own demand. Of the remaining six patients, EGF administration was terminated in three (1.7%) due to local infection not dependent on the baseline ulcer infection status and in three (1.7%) because of adverse effects. Four patients died of various causes, all unrelated to their diabetic foot wound.

Discussion

Intralesional EGF has been available as a medication in Turkey since 2012, and, to our knowledge, this is the first report providing a clinical assess-

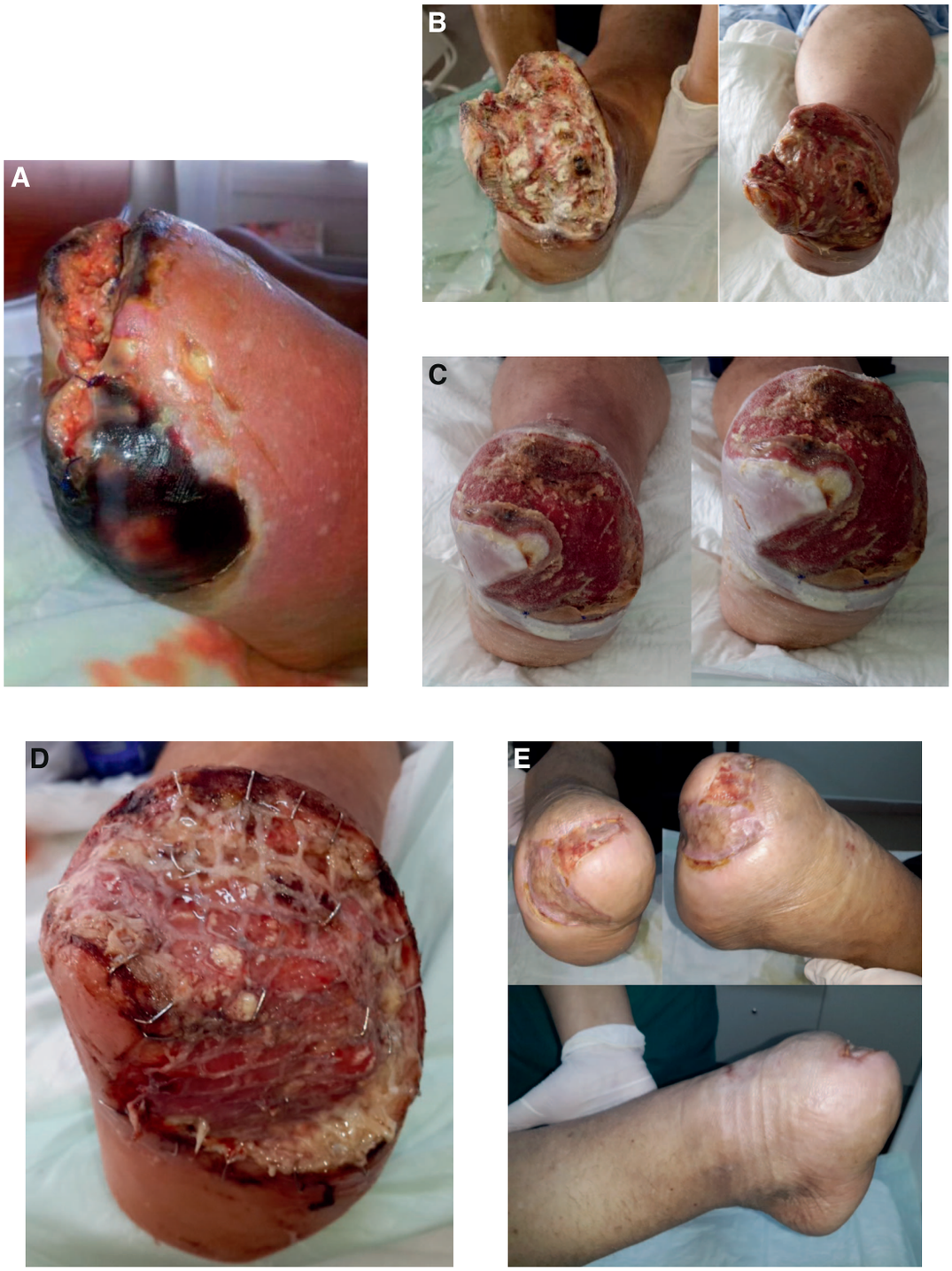

Figure 4. Clinical views of a diabetic foot ulcer before treatment (A), after surgical debridement (B), after the sixth (left) and ninth (right) intralesional epidermal growth factor applications (C), after autologous skin graft (D), and during follow-up (E).

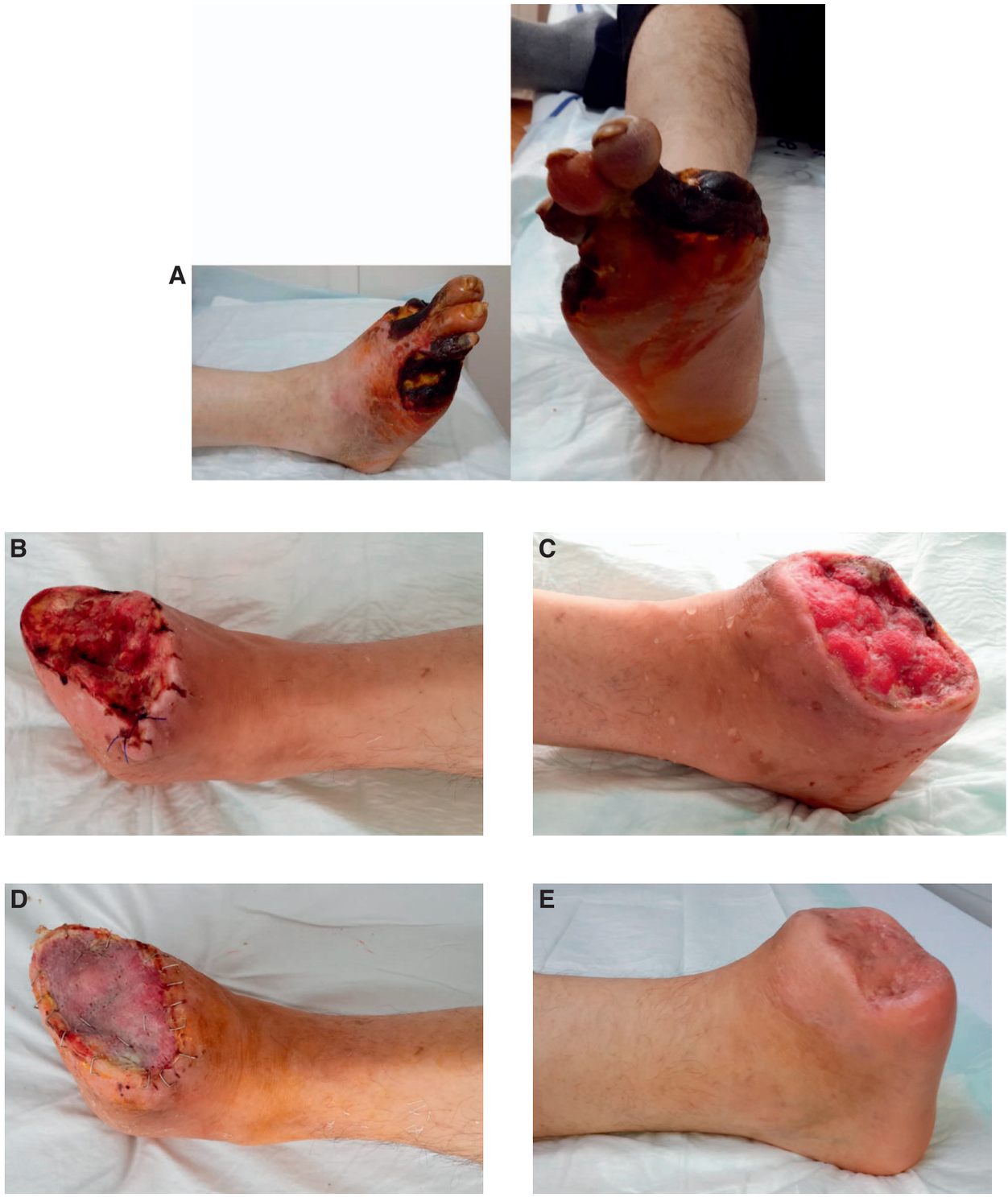

Figure 5. Clinical views of a diabetic foot ulcer before treatment (A), after surgical debridement and minor amputation (B), after the 15th intralesional epidermal growth factor application (C), after autologous skin graft (D), and during follow-up (E).

Table 4. Outcomes After Intralesional EGF Administration in 44 Patients with Renal Failure

	Patients (No. [%])				
Outcome	No Response	Minimal Response	Partial Response	Complete Response	Total
Wound closure with only EGF administration	0	0	2	13	15 (34)
Wound closure by simple surgical suture with EGF administration	0	1	4	1	6 (14)
Wound closure by skin graft with EGF administration	0	0	1	12	13 (30)
Wound size decreased (but without closure) only with EGF administration	1	2	1	0	4 (9)
Recurrent infection	2	0	1	0	3 (7)
Major amputation	1	0	0	1	2 (4)
Death	0	0	0	1	1 (2)
Total	4 (9)	3 (7)	9 (20)	28 (64)	44 (100)

Note: See the Materials and Methods section for definitions of the response categories.
Abbreviation: EGF, epidermal growth factor.

ment of its efficacy and safety for diabetic foot ulcers outside Latin America. In this study, we aimed to analyze the outcomes of patients with diabetic foot ulcers treated with intralesional EGF. Physicians from the enrolled centers completed the forms designed for the study.

Optimally managing diabetic foot ulcers requires a combination of various treatment modalities. Because this is not a comparative study but one performed with conventional therapies used together with EGF as a new adjuvant treatment option, we believe that it is best to evaluate the outcomes of the study in two different ways. The first evaluation was based on the percentage of healthy granulation tissue in the base of the ulcer at the end of EGF treatment, the same method described in previous clinical studies with EGF. There are currently few studies on this topic, and all have evaluated the results of intralesional EGF treatment in the same way as discussed previously herein. In a phase 2 study, Fernandez-Montequin et al[16] compared EGF doses of 75 μg (n = 23) and 25 μg (n = 18) and found a greater and faster complete response with the higher dose (83% versus 61%). In another double-blind randomized and multicenter phase 2 study, Fernandez-Montequin et al[15] compared two different doses of EGF (75 and 25 μg) with placebo. The rates of complete response after 8 weeks of treatment were 87% with 75 μg of EGF, 73% with 25 μg of EGF, and 58% with placebo. In a postmarketing study performed in Cuba, both 75- and 25-μg doses (only in ulcers <20 cm^2) were evaluated. Among 1,835 total treatment courses, the complete response rate was 75.9%.[17] In a study

Table 5. Logistic Regression Analysis of the Factors Affecting Complete Response and Wound Closure with Only Intralesional EGF Administration

Outcome and Factor	*P* Value	Odds Ratio	95% Confidence Interval
Complete response			
Previous osteomyelitis	.026	2.87	1.14–7.26
Duration of diabetic foot ulcer (>75 d)	.003	3.05	1.46–6.41
Wound closure with only intralesional EGF administration			
Previous osteomyelitis	.02	2.89	1.19–7.03
Male sex	.012	3.19	1.29–7.84
Age ≥56 years	.003	4.04	1.60–10.18

Abbreviation: EGF, epidermal growth factor.

Table 6. Results of a Univariate Analysis of Factors Affecting Wound Closure in Patients Treated with Only Intralesional Epidermal Growth Factor Administration

		Wound Closure Without Additional Therapeutic Interventions (No.)		
Factor	Patients (Total No.)	Yes	No	*P* Value
Sex	174			
Female	48	32	16	.002
Male	126	49	77	
Smoking	153			
No	95	53	42	.011
Yes	58	19	39	
Previous foot ulcer	164			
No	69	43	26	.003
Yes	95	35	60	
Previous vascular surgery	156			
No	121	62	59	.02
Yes	35	9	26	
Peripheral vascular disease	174			
Grade 1	79	46	33	.035
Grade 2	62	24	38	
Grade 3	33	11	22	
Wound depth	174			
Grade 1	45	34	11	<.001
Grade 2	80	33	47	
Grade 3	49	14	35	
Infection	174			
Grade 1	40	28	12	.001
Grade 2	34	20	14	
Grade 3	78	26	52	
Grade 4	22	7	15	
Osteomyelitis	169			
No	112	65	47	.001
Yes	57	16	41	
Wagner classification	174			
Grade 1	31	26	5	<.001
Grade 2	43	23	20	
Grade 3	68	26	42	
Grade 4	26	6	20	
Grade 5	1	0	1	
Negative pressure wound therapy	161			
No	112	62	50	.007
Yes	49	16	33	
Age				
<56 years	53	33	20	.01
≥56 years	121	48	73	
Hemoglobin A_{1c}	134			
<7%	66	38	28	.038
≥7%	68	27	41	
Wound size	170			
<14 cm^2	82	48	34	.006

Table 7. Adverse Effects Associated with Intralesional Epidermal Growth Factor Administration

Adverse Effect	Patients (No. [%])
Chills and shivering	66 (37.9)
Nausea	40 (22.9)
Pain at application site	37 (21.3)
Vomiting	12 (6.9)
Hypotension	8 (4.6)
Infection at the treated site	8 (4.6)
Burning sensation	6 (3.4)
Fever	4 (2.3)
Hypertension	4 (2.3)
Erythema	4 (2.3)
Chest pain	3 (1.7)
Palpitation	3 (1.7)
Syncope	3 (1.7)
Respiratory distress	1 (0.6)
Dizziness	1 (0.6)
Necrosis	1 (0.6)

performed in Argentina, the rates of complete response and complete wound closure after 75-μg intralesional EGF treatment were 70.3% and 69.2%, respectively.[18] Another study performed with 75 μg of intraregional EGF found a rate of total wound closure of 58.4%.[19] Among these five studies, a common feature was the duration of EGF treatment for 8 weeks (24 applications).

In Turkey, only 75-μg vials of EGF are available, so we administered this dose to all of the patients in this study. We found that the rates of complete response and wound closure after EGF injection alone were 66.7% and 46.6%, respectively. These rates are lower than those in the previously reported studies. The main difference was that in the previous studies the total duration of EGF administration was 8 weeks in all patients, whereas in the present study the median administration duration was 4 weeks. In the present study, when the wound had adequate granulation tissue, the enrolling physicians could select either simple surgical suture or skin graft for wound closure and could terminate the EGF therapy earlier than in the previous studies. In the second evaluation method we took into account not only the presence of granulation tissue but also total wound closure, categorized by the type of surgical procedure. The number of patients achieving total wound closure at the end of EGF treatment was 81 (46.6%), but this number reached 146 (83.9%) when EGF was combined with one of the surgical procedures (Table 3). This treatment success rate is higher than the other reported case series, except for the phase 3 study.[15] Thus in patients with adequate granulation tissue at the ulcer site, performing a surgical wound closure procedure may be a cost-effective approach compared with waiting for a response to EGF therapy alone.

This study also differed from the previously published ones in that we classified the enrolled patients according to the PEDIS classification. This enabled us to observe the potential effects of factors other than foot lesions (eg, circulatory failure, wound depth, and presence of systemic infection) on treatment success. Most of these patients had evidence of limb vascular insufficiency, deep wounds, and severe infections. Although our patient population was similar to previously studied groups, the 2.9% rate of major lower-limb amputation was lower than the 9.3% in a postmarketing study performed by Yera-Alos et al,[17] the 10.7% reported by Guillermo et al,[18] and the 9.38% reported by Velazquez et al.[20] In a comparative study, Gonzalez-Acosta et al[21] found that compared with standard wound therapy, intralesional EGF added to standard therapy was associated with a lower rate of major amputation (26.7% versus 8.3%). A similarly designed study reported a reduction in major amputations from 43.1% to 8.1%.[22] The reported rates of lower-extremity amputation for diabetic patients with a severe foot ulcer are generally 14% to 25%,[23-26] and approximately 60% of amputations are preceded by infected ulcers.[27] The present study suggests that initiation of EGF administration after immediate control of the infection might substantially lower that rate. When the ulcer was infected, we controlled it by a routine of debridement or minor amputations and antibiotic drug therapy; when there was a clean wound surface we initiated EGF injections along with the antibiotic drug therapy. We could not initiate EGF injections in patients who underwent major amputation due to uncontrolled infection. Of note, despite the fact that most of these patients (57.4%) had high-grade infections (grades 3 and 4), the amputation rate in this study was notably low.

In other research on EGF, patients with renal failure were excluded from the study, whereas we included these patients. The data from patients with renal failure obtained in this study were the first in the literature. Renal failure did not have a negative effect on treatment results with EGF. In addition, in these patients, the rate of adverse effects due to EGF treatment was similar to that in the other patients. The number of patients with renal failure

(n = 44) achieving complete response at the end of intralesional EGF treatment was 28 (64%), and this number reached 34 (78%) when EGF was combined with one of the surgical procedures (Table 4). As a result, intralegional EGF administration seems to be a safe medication in patients with renal failure.

During follow-up, the ulcer reccurred at the treatment site in 12 patients (8.1%). Yera-Alos et al[17] reported a recurrence rate of 5% in their postmarketing study. Recurrence of the ulcer in patients with diabetes is usually due to inadequate preventive care of the foot by the patient. Unfortunately, we were not able to obtain any information about preventive care in the present patients because the data form did not require recording of this information.

In the present patients, there were 201 adverse effects of 16 different types that occurred in 97 patients (55.7%). Most of these patients experienced mild adverse effects, such as chills, shivering, nausea, and pain or a burning sensation at the application site. The rate of adverse effects was lower than the rates in the phase 3 studies (69.8%)[28] or that of Yera-Alos et al (46%).[17] Severe adverse effects in the present patients included infection, hypotension, syncope, and respiratory distress. Both the mild and severe adverse effects occurred shortly after the EGF was injected and (except for infection) lasted 1 to 2 hours. The half-life of EGF in the wound area is short, as it is degraded within approximately 2 hours.[29] There is a lack of information on the mechanisms of general adverse effects of intralesional EGF therapy in the literature. It is possible that infections of the wound site might be related to a lack of adequate sterilization of the application site.

Because the measurement of baseline granulation tissue before EGF administration is lacking in this study, we could not compare the difference before and after EGF treatment. It is a limitation of the present study.

Conclusions

The potential role of growth factors in healing diabetic foot ulcers has been studied for decades. Epidermal growth factor has a direct effect on wound healing. This study provides the first data on the efficacy and safety of intralesional EGF for treating diabetic foot ulcers outside of Latin America. The results of this study may change the approach to current algorithms for managing diabetic foot ulcers. This trial suggests that in patients with a diabetic foot ulcer receiving standard care, the addition of intralesional EGF (after infection control) provides high healing and low amputation rates. In patients with adequate granulation tissue at the ulcer site, performing surgical wound closure procedures may accelerate wound healing over waiting for a response to EGF alone.

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References

1. Cavanagh PR, Lipsky BA, Bradbury AW, et al: Treatment for diabetic foot ulcers. Lancet **366:** 1725, 2005.
2. Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, et al: The global burden of diabetic foot disease. Lancet **366:** 1719, 2005.
3. Singh N, Armstrong DG, Lipsky BA: Preventing foot ulcers in patients with diabetes. JAMA **293:** 217, 2005.
4. Armstrong DG, Cohen K, Courric S, et al: Diabetic foot ulcers and vascular insufficiency: our population has changed, but our methods have not. J Diabetes Sci Technol **5:** 1591, 2011.
5. Lipsky BA, Berendt AR, Cornia PB, et al: 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis **54:** e132, 2012.
6. Schaper NC, Apelqvist J, Bakker K: The international consensus and practical guidelines on the management and prevention of the diabetic foot. Curr Diab Rep **3:** 475, 2003.
7. Armstrong DG, Wrobel J, Robbins JM: Are diabetes-related wounds and amputations worse than cancer? Int Wound J **4:** 286, 2007.
8. Bus SA, Valk GD, Van Deursen, et al: The effectiveness of footwear and offloading interventions to prevent and heal foot ulcers and reduce plantar pressure in diabetes: a systematic review. Diabetes Metab Res Rev **24** (suppl 1)**:** S162, 2008.
9. Cohen S: Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. J Biol Chem **237:** 1555, 1962.
10. Tsang MW, Wong WK, Hung CS, et al: Human epidermal growth factor enhances healing of diabetic foot ulcers. Diabetes Care **26:** 1856, 2003.
11. Berlanga-Acosta J: Diabetic lower extremity wounds: the rationale for growth factors-based infiltration treatment. Int Wound J **8:** 612, 2011.
12. Gibbs S, Silva Pinto AN, Murli S, et al: Epidermal growth factor and keratinocyte growth factor differentially regulate epidermal migration, growth, and differentiation. Wound Repair Regen **8:** 192, 2000.
13. Schaper NC: Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. Diabetes Metab Res Rev **20** (suppl 1)**:** S90, 2004.

14. Lipsky BA, Peters EJ, Senneville E, et al: Expert opinion on the management of infections in the diabetic foot. Diabetes Metab Res Rev **28** (suppl 1)**:** S163, 2012.
15. Fernandez-Montequin JI, Betancourt BY, Leyva-Gonzalez G, et al: Intralesional administration of epidermal growth factor-based formulation (Heberprot-P) in chronic diabetic foot ulcer: treatment up to complete wound closure. Int Wound J **6:** 67, 2009.
16. Fernandez-Montequin JI, Infante-Cristia E, Valenzuela-Silva C, et al: Intralesional injections of Citoprot-P (recombinant human epidermal growth factor) in advanced diabetic foot ulcers with risk of amputation. Int Wound J **4:** 333, 2007.
17. Yera-Alos IB, Alonso-Carbonell L, Valenzuela-Silva CM, et al: Active post-marketing surveillance of the intralesional administration of human recombinant epidermal growth factor in diabetic foot ulcers. BMC Pharmacol Toxicol **14:** 44, 2013.
18. Guillermo G, Calvagno M, Tolstano A, et al: Treatment of severe diabetic foot ulcers with recombinant epidermal growth factor (Heberprot-P): retrospective analysis of the obtained in Argentina [in Spanish]. Rev Argentina Cirugia Cardiovasc **10:** 153, 2012.
19. Valenzuela-Silva CM, Tuero-Iglesias AD, Garcia-Iglesias E, et al: 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 **36:** 210, 2013.
20. Velazquez W, Vales A, Curbelo W: Impact of epidermal growth factor on the treatment of diabetic foot ulcers. Biotecnologia Aplicada **27:** 136, 2010.
21. Gonzalez-Acosta S, Calana-Gonzales-Posada B, Marrero-Rodriguez I, et al: Clinical evolution of diabetic foot treatment with Heberprot-P or with the conventional method [in Spanish]. Rev Cubana Angiol Cirugía Vascular **11:** 11, 2011.
22. 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 [in Spanish]. Spanish J Surg Res **XIV:** 21, 2011.
23. Blanes JI: Consensus document on treatment of infections in diabetic foot. Rev Esp Quimioter **24:** 233, 2011.
24. Ertugrul BM, Oncul O, Tulek N, et al: A prospective, multi-center study: factors related to the management of diabetic foot infections. Eur J Clin Microbiol Infect Dis **31:** 2345, 2012.
25. Ghanassia E, Villon L, Thuan Dit Dieudonne JF, et al: Long-term outcome and disability of diabetic patients hospitalized for diabetic foot ulcers: a 6.5-year follow-up study. Diabetes Care **31:** 1288, 2008.
26. Winkley K, Stahl D, Chalder T, et al: Risk factors associated with adverse outcomes in a population-based prospective cohort study of people with their first diabetic foot ulcer. J Diabetes Complications **21:** 341, 2007.
27. Lipsky BA: Medical treatment of diabetic foot infections. Clin Infect Dis 3**9** (suppl 2)**:** S104, 2004.
28. Fernandez-Montequin JI, Valenzuela-Silva CM, Diaz OG, et al: Intra-lesional injections of recombinant human epidermal growth factor promote granulation and healing in advanced diabetic foot ulcers: multicenter, randomised, placebo-controlled, double-blind study. Int Wound J **6:** 432, 2009.
29. Gomez-Villa R, Aguilar-Rebolledo F, Lozano-Platonoff A, et al: Efficacy of intralesional recombinant human epidermal growth factor in diabetic foot ulcers in Mexican patients: a randomized double-blinded controlled trial. Wound Repair Regen **22:** 497, 2014.