Diabetes, Metabolic Syndrome and Obesity

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CASE SERIES

Effects of Recombinant Human Granulocyte/Macrophage Colony-Stimulating Factor on Diabetic Lower Extremity Ulcers: Case Series of Nine Patients

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Background: Diabetic lower extremity ulcer, including diabetic foot ulcer (DFU) and leg ulcer, is one of the refractory complications of diabetes, the treatment of which is challenging, expensive, and lengthy. Recombinant Human Granulocyte/Macrophage Colony-stimulating Factor (rhGM-CSF) is an immunomodulatory cytokine that has been mainly applied in the treatment of hematological diseases. Clinical evidence regarding GM-CSF in the treatment of diabetic lower extremity ulcers is limited. This study is the first case series that investigates the repurpose effects of rhGM-CSF on diabetic ulcer healing in real clinical practice.

Methods: Nine patients diagnosed with diabetes and refractory lower extremity ulcer (ulcer duration ≥2 weeks) were included from September 2021 to February 2023 in the Division of Endocrinology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Patients with Wagner grade ≥4 and SINDAD ≥5 were excluded. The included subjects were treated with rhGM-CSF plus standard of care (SOC) including glycemic control, foot care education, debridement of necrotic tissues, topical wound dressings, offloading, and infection control when necessary. The observation endpoint was complete epithelialization. Their clinical manifestations, laboratory tests, and therapeutic effects were extracted and analyzed.

Results: The case series included 9 cases aged from 29 to 80 years and all the patients were male. Seven of 9 patients presented neuropathic ulcer. Only one case showed non-infected ulcer from tissue samples and one case presented ankle brachial index (ABI) <0.9. It was observed that the ulcer areas among these 9 patients gradually declined throughout the whole treatment period with the average healing velocity 0.32 ± 013 cm^2/day and the mean time to complete healing 16.0 ± 3.7 days. The relative area (percentage of initial ulcer area) decreased to 66.7 ± 13.0% on average after the first treatment. Ulcers in all the 9 patients achieved complete epithelialization after 4–8 times treatments.

Conclusion: The case series suggests rhGM-CSF as a promising therapeutic strategy for the treatment of diabetic ulceration. More robust data from randomized controlled trials are required to further evaluate its clinical efficacy.

Keywords: recombinant human granulocyte/macrophage colony-stimulating factor, diabetic lower extremity ulcer, diabetic foot ulcer

Introduction

Diabetic lower extremity ulcer, including diabetic foot ulcer (DFU) and leg ulcer, is a common, complex, disabling, and costly complication of diabetes.[1] It is estimated that 19–34% of patients with diabetes develop a foot ulcer in their lifetime.[1] Patients with DFUs are at increased risk for recurrence, and the rates at 1, 3, and 5 years were 40%, 60%, and 65%, respectively, according to 19 compatible studies.[1] In addition, diabetic ulcers also cause a huge socio-economic burden worldwide.[2] It was reported that direct costs for diabetes in the USA were $237 billion in 2017, about one-third of which was attributable to DFU.[3]

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The standard of care (SOC) of diabetic ulcers consists of blood glucose and infection control, debridement of necrotic tissues, revascularization, mechanical offloading, dressing change, and foot care education.[4] However, the effect of these multidisciplinary approaches on wound healing could not completely meet clinical needs.[5,6] It has been reported that the median healing time of DFUs was 76 days for male patients and 117 days for female patients, according to a clinical study among 1,488,201 hospitalized patients in China.[5] In addition, the healing percentage of diabetic ulcer in our hospital was approximately 70% after 1 month when patients were only undertaken standard treatment (unpublished data). Therefore, some new adjunctive therapies are needed to promote the healing of DFUs.

Hemopoietic colony stimulating factors (CSFs), including Granulocyte-CSF (G-CSF) and Granulocyte/Macrophage CSF (GM-CSF), are glycoproteins that regulate blood cell proliferation and differentiation.[7] Accordingly, G- and GM-CSF are widely used in the treatment of blood system diseases.[8]

G-CSF could promote the release of neutrophil endothelial progenitor cells from the bone marrow, induce the differentiation of neutrophils, and improve the defective neutrophil functions.[7] Thus, this growth factor is applied in patients with diabetic foot infection and has been well studied in plenty of clinical studies.[9]

GM-CSF is mainly derived from immune cells and bone marrow cells,[8] which has been identified to induce the proliferation, differentiation, and maturation of hematopoietic precursor cells in the myeloid and erythroid lineages.[10] Different from G-CSF, multiple in-vitro studies indicated that GM-CSF could modulate a variety of cellular functions that are essential to wound healing, including the migration of epithelial cells, activation of neutrophils and monocytes/macrophages, proliferation of keratinocytes, and transition of fibroblast phenotype.[11–13] Some animal studies indicate that GM-CSF treatment enhances wound repair in various clinical conditions such as corneal wound,[14] vocal fold injury,[15] and skin ulcer with normoglycemia.[16,17] Moreover, a few clinical studies have applied GM-CSF to treat human chronic skin ulcers.[18–22] A systematic review in 2011 disclosed that rhGM-CSF therapy through both topical administration and subcutaneous injection could promote wound repair for burn wounds, chronic leg ulcers, and leprosy ulcers.[21]

Currently, there are few studies investigating the clinical effects of GM-CSF on DFUs. Only one case report in 2018 indicated that intra-lesional injection of GM-CSF could promote the healing of diabetic neuropathic ulcer.[23] Thus, the present case series aims to investigate the therapeutic outcomes of Recombinant Human GM-CSF (rhGM-CSF) in the healing of refractory diabetic ulcers.

Methods

This is a formal clinical case series of 9 cases of patients with type 2 diabetes mellitus (T2DM) suffering from refractory lower extremity ulcers including foot ulcers and leg ulcers. Adult patients aged 18–80 years old were eligible for the study if the ulcer duration was more than two weeks.[24] Key exclusion criteria included pregnancy, Wagner grade ≥4 or SINDAD ≥5, and ulcers not on the leg or feet. Detailed information including age, sex, comorbidities, and biochemical tests were collected. The endpoint of the observation is the complete epithelialization of the ulcers. Our study complies with the Declaration of Helsinki.

The severity of ulcers was evaluated by Wagner grade and SINBAD score.[25,26] Ulcer infection was assessed by PEDIS score[27] and culture of wound tissue. The vascular assessment was evaluated using ankle brachial index (ABI), lower extremity arterial and venous ultrasound, and computed tomography angiography (CTA). Neuropathy was defined by the reduced nerve conduction velocity assessed by electromyogram and the abnormalities of pinprick, vibration perception, pressure perception, and temperature perception.[28]

Patients all received rhGM-CSF and SOC therapy including glycemic control, debridement of necrotic tissues, topical wound dressings, offloading, antibiotic therapy, and foot care education. The rhGM-CSF was topically administrated on the surface of ulcers and then covered with a sterile wound dressing every 3 days. The amount of rhGM-CSF was determined according to the ulcer area (2.0 μg/cm^2).

The ulcers were clinically evaluated every 3 days by two physicians independently. The ulcer area was calculated using the ImageJ software (Version 1.53).[29] We defined the initial ulcer area as 100% and calculated the relative ulcer area as a percentage of the initial ulcer area.

Case Description

Cases are individually described below.

Case 1

A 47-year-old male patient presented with a case of T2DM for 2 years, who was on oral hypoglycemic drugs and insulin therapy. He developed a red and swollen ulcer at the left first toe two weeks ago. The patient was treated with debridement and conventional dressing changes in a local hospital, but the ulcer deteriorated. The patient then presented to our division for further treatment.

His medical history revealed hypertension, stroke, chronic kidney disease (CKD) stage 4, and retinopathy. He was also a smoker. The body weight was 67 kg with a body mass index (BMI) 24.61 kg/m^2. On admission, the patient was conscious with a body temperature of 36.6°C, pulse rate of 88 beats/min, and blood pressure of 122/81mmHg. The level of HbA1c was 7.21%, and albumin-to-creatinine ratio (ACR) was 2066.4 μg/mg (Table 1). The ulcer appeared swollen without purulent discharge, the size of which was 4.17 cm^2. The grade of ulcer was Wagner 1, SINBAD 3, and PEDIS 2. Vascular Doppler ultrasound of the lower extremities indicated bilateral multiple atherosclerotic plaques. The left ABI was 1.16, and the right ABI was 1.19. The protective sensation in both feet was diminished. A tissue sample from the ulcer was collected and *pseudomonas aeruginosa* was identified.

The patient was recommended to decrease physical activities. An inflatable wedge was applied when he was in bed. After thorough debridement and antibiotic therapy (Moxifloxacin Hydrochloride), the bacterial culture turned negative. Subsequently, this patient received rhGM-CSF treatment with an initial dose of 8.34 μg. After 5 rhGM-CSF treatments along with SOC, the ulcer achieved complete epithelialization (Figure 1).

Case 2

A 56-year-old male patient presented with a two-year history of T2DM and was treated with insulin since the diagnosis of diabetes. Seven months ago, the patient developed double heel ulcers with obvious inflammatory manifestations, including redness, swelling, heat, and tenderness. After 5-month SOC including anti-infection, debridement, and dressing changes in a local hospital, the right one completely healed, while the ulcer on the left heel did not respond well to the treatment. The patient was then transferred to our division.

He admitted to a history of cigarette smoking, hypertension, cardiovascular disease, and CKD and to be under regular dialysis treatment. His weight was 70 kg, and BMI was 24.50 kg/m^2. On admission, the body temperature of this patient was 36.5°C, with pulse rate of 91 beats/min and blood pressure of 146/80mmHg. The glycemia of this patient was well controlled with HbA1c 5.70%. The level of ACR was 12,751.2 μg/mg, and estimated glomerular filtration rate (eGFR) was only 21.2 mL/min/1.73m^2 (Table 1). The ulcer size was 7.38 cm^2, which appeared swollen, red, odorless, and achy. The grade of ulcer was Wagner grade 1, SINBAD 4, and PEDIS score 2. Blood perfusion in the lower extremities appeared normal according to vascular Doppler ultrasound and ABI detection (left 1.16, right 1.15). Inflammatory biomarkers high-sensitivity C-reactive protein (hsCRP) and erythrocyte sedimentation rate (ESR) were markedly elevated. No bacteria was identified from the microbial culture.

The patient was stationary in bed, and an inflatable cushion was used to offload pressure. Ceftriaxone was used for 5 days and the inflammatory biomarkers returned to the normal range. The initial dose of rhGM-CSF was 14.76 μg and the ulcer achieved complete epithelialization after 8 rhGM-CSF treatments (Figure 2).

Case 3

A 52-year-old male patient was admitted to our department presented with a two-year history of dry mouth and polydipsia accompanied by a skin ulcer of about 4.79 cm^2 on the right heel for one month. The ulcer extended deep to the bone with exposed subcutaneous tissue and tendon. He was treated with conventional dressing changes in a local hospital, but the ulcer area did not reduce. The patient then presented to our division.

He has a medical history of retinopathy and was also a smoker. His weight was 85 kg, and BMI was 27.76 kg/m^2. The patient had a fever on admission with a body temperature of 37.6°C, pulse rate of 92 beats/min, and blood pressure of

Table 1 Characteristics and Laboratory Findings on Admission

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Reference Range
Age (years)	47	56	52	59	74	69	65	29	80	
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male	
BMI	24.61	24.50	27.76	27.08	26.3	25.2	22.2	22.1	27.5	
Duration of T2DM (years)	2	2	2	14	16	5	13	3	20	
Duration of DFU (days)	14	210	30	15	18	120	28	30	180	
Comorbidities										
Hypertension	+	+	–	–	–	+	+	–	+	
Cardiovascular disease	–	+	–	–	–	+	–	–	–	
Stroke	+	–	–	–	–	–	–	–	–	
CKD stage	4	5	–	2	3	3	2	2	3	
Retinopathy	+	/	+	+	+	/	–	–	–	
Laboratory data										
WBC (10^9/L)	10.40	2.59	14.18	5.55	8.49	9.40	5.51	6.28	8.48	3.5 ~ 9.5
Hemoglobin (g/L)	132.0	87.0	107.0	143.0	130.0	131.0	134.0	144.0	129.0	130.0~175.0
Platelet (10^9/L)	211.0	88.0	584.0	204.0	265.0	338.0	243.0	277.0	213.0	125.0~350.0
ALT (U/L)	57	19	36	16	12	13	9	9	21	≤ 41
AST (U/L)	30	30	43	12	20	13	10	14	22	≤ 40
LDH (U/L)	222	310	256	162	272	220	172	266	239	135 ~ 225
Cr (μmol/L)	92	199	62	90	116	100	89	51	110	59 ~ 104
eGFR(mL/min/1.73m^2)	57.0	21.2	108.4	79.1	53.1	65.9	78	138.1	54.3	≥ 90
ACR (μg/mg)	2066.4	12,751.2	13.2	13.1	117.5	142.5	9.3	586.6	29.1	< 30
UA (mmol/L)	325.3	142.0	123.0	297.0	448.0	369.0	271.0	334.0	394.0	202.3~416.5
hsCRP (mg/L)	2.1	7.8	78.1	0.9	20.9	11.5	4.7	5.7	2.9	< 1
ESR (mm/H)	27	48	86	10	40	39	23	20	7	0~15
cTnI (pg/mL)	5.0	502.4	1.9	2.0	7.2	4.6	1.9	1.9	8.3	≤ 34.2
NTproBNP (pg/mL)	325	70,000	49	80	/	66	45	/	365.1	< 486
TC (mmol/L)	3.38	3.47	3.78	3.04	3.94	3.85	4.31	5.95	3.98	< 5.18
TG (mmol/L)	1.45	1.12	1.62	1.13	1.08	2.39	1.91	14.23	8.68	< 1.70
HbA1c (%)	7.21	5.70	13.60	5.80	10.00	7.40	10.30	12.70	6.30	4.30 ~ 6.10
Initial ulcer area(cm^2)	4.17	7.38	3.84	3.50	2.50	10.91	6.24	6.74	2.63	
Ulcer location	Left first toe	Left heel	Right heel	Left second toe	Sole of right foot	Right lower limb	Dorsum of left foot	Right lower limb	Right first toe	
Vascular evaluation										
ABI (left)	1.16	1.16	0.96	1.04	1.05	0.98	0.98	1.04	0.75	0.91 ~ 1.30
ABI (right)	1.19	1.15	1.23	1.25	1.18	1.04	1.06	1.01	0.50	
Wound evaluation										
SINBAD	3	4	4	2	3	3	3	3	3	
Wagner	1	1	3	1	2	3	2	2	1	
PEDIS	2	2	3	2	2	3	2	3	2	

Neuropathic ulcer	+	+	+	+	+	/	+	–	+	
Secretion culture	Pseudomonas aeruginosa	Normal skin flora	Klebsiella pneumoniae	Acinetobacter baumannii	Staphylococcus aureus	Staphylococcus aureus	Coagulase negative staphylococcus	Staphylococcus aureus	Candida parapsilosis	
Antibiotics	Moxifloxacin Hydrochloride	Ceftriaxone	Biapenem	Sulbactam / Cefoperazone	Linezolid	Teicoplanin, Sulbactam / Cefoperazone	Ceftriaxone	Teicoplanin	Piperacillin Sodium and Tazobactam Sodium	

Notes: +, yes; -, no; /, not available.

Abbreviations: ABI, ankle brachial index; ACR, albumin-to-creatinine ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cr, creatine; hsCRP, high-sensitivity C-reactive protein; cTnI, cardiac troponin I; DFU, diabetic foot ulcer; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; HbA1c, Hemoglobin A1c; LDH, lactate dehydrogenase; L-PRF; leukocyte-and platelet-rich fibrin; NTproBNP, N-terminal pro-b-type natriuretic peptide; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; UA, urine acid; WBC, white blood cell.

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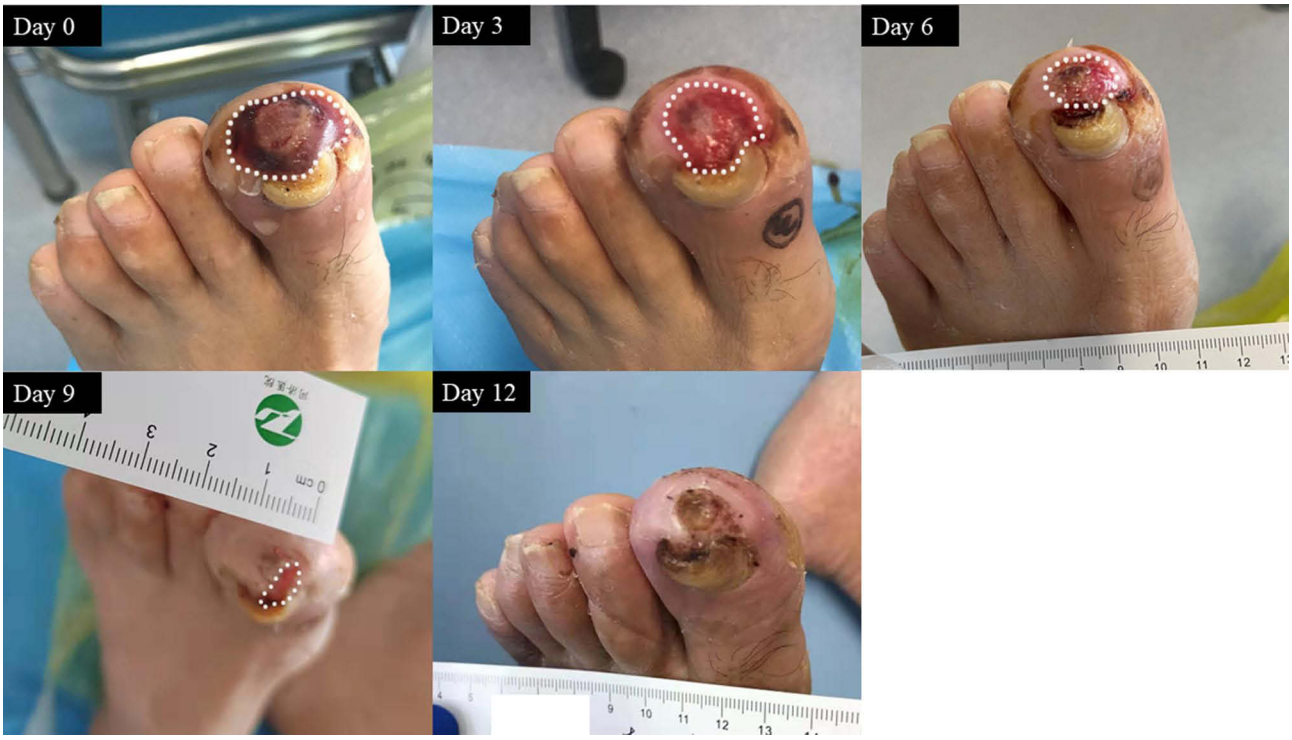

Figure 1 Representative images of the healing process in case 1.

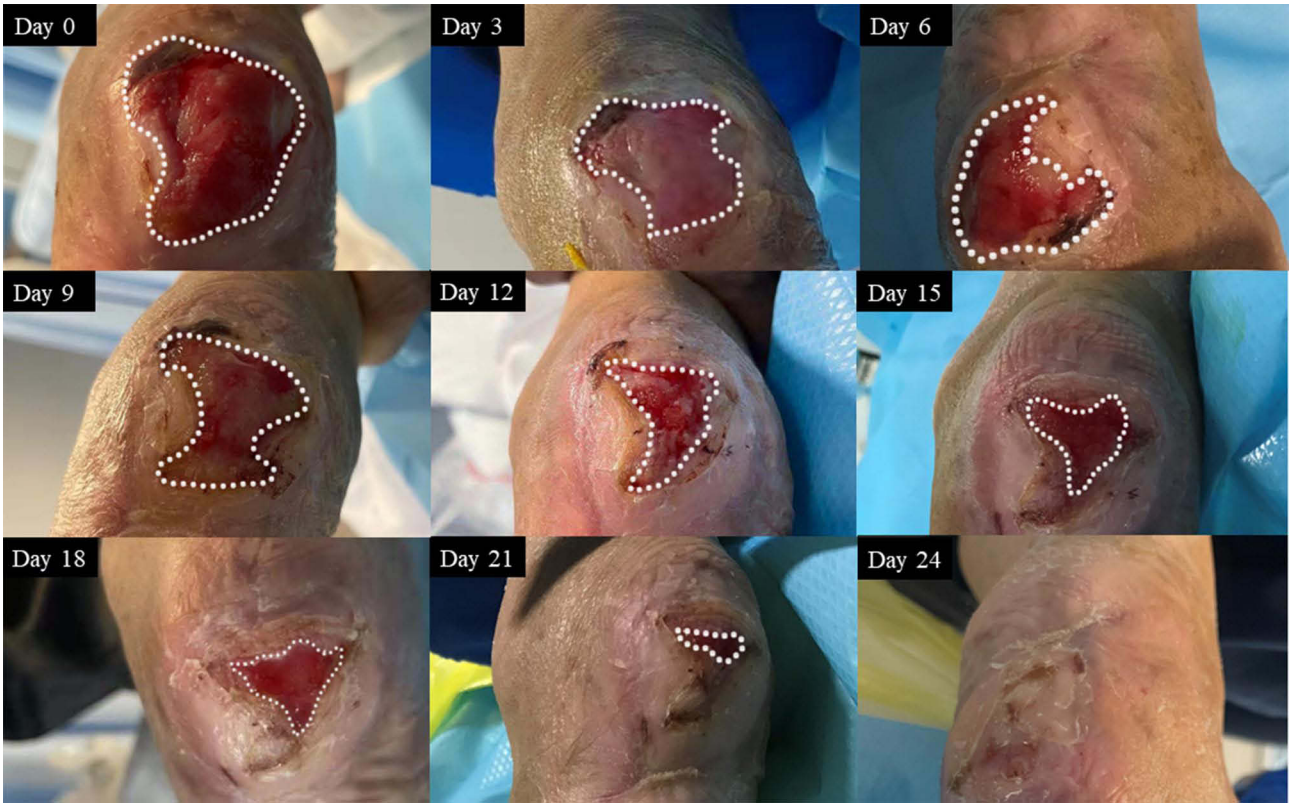

Figure 2 Representative images of the healing process in case 2.

102/71mmHg. This patient had a poor glucose control with HbA1c 13.60%. The ulcer size was 3.84 cm^2. The surrounding skin of the ulcer showed inflammatory manifestations, including redness, tenderness, and increased temperature. His ulcer was neuropathic, and the grade of ulcer was Wagner 3, SINBAD 4, and PEDIS 3. No abnormalities were identified by vascular Doppler ultrasound, and ABI was in the normal range. Markedly elevated ESR and hsCRP indicated severe infection. *Klebsiella pneumoniae* was identified from the bacterial culture (Table 1).

The patient was treated with surgical debridement, vacuum sealing drainage (VSD), and biapenem for infection control. An inflatable cushion was used for pressure offloading. Subsequently, the patient received rhGM-CSF treatment with an initial dose of 7.68 μg and the ulcer healed after 6 treatments (Figure 3).

Case 4

A 59-year-old male patient had been diagnosed with T2DM for 14 years who was on metformin and insulin therapy. He presented with an ulcer that appeared on the left second toe for half a month. The ulcer size was 3.50 cm^2, with no signs of infection. He admitted to a history of cigarette smoking, CKD, and retinopathy. His weight was 82 kg, and BMI was 27.08 kg/m^2. On admission, the patient's body temperature was 36.8°C with pulse rate of 84 beats/min and blood pressure of 150/106 mmHg. As shown in Table 1, the level of HbA1c was 5.80% with normal liver and kidney function. The ulcer type was neuropathic, and the ulcer evaluation was Wagner 1, SINBAD 2, and PEDIS 2. Vascular Doppler ultrasound of the lower extremities indicated bilateral atherosclerotic plaques and ABI indicated adequate perfusion (Table 1). Although inflammatory biomarkers of ESR and hsCRP were shown to be within the normal range, *Acinetobacter baumannii* was identified in the microbial culture test.

An inflatable wedge was applied for pressure offloading when the patient was in bed. After antibiotic therapy (Sulbactam/Cefoperazone), the bacterial culture turned negative. Then, this patient received rhGM-CSF treatment with an initial dose of 7.0 μg. After 4 rhGM-CSF treatments, the ulcer achieved complete epithelialization (Figure 4).

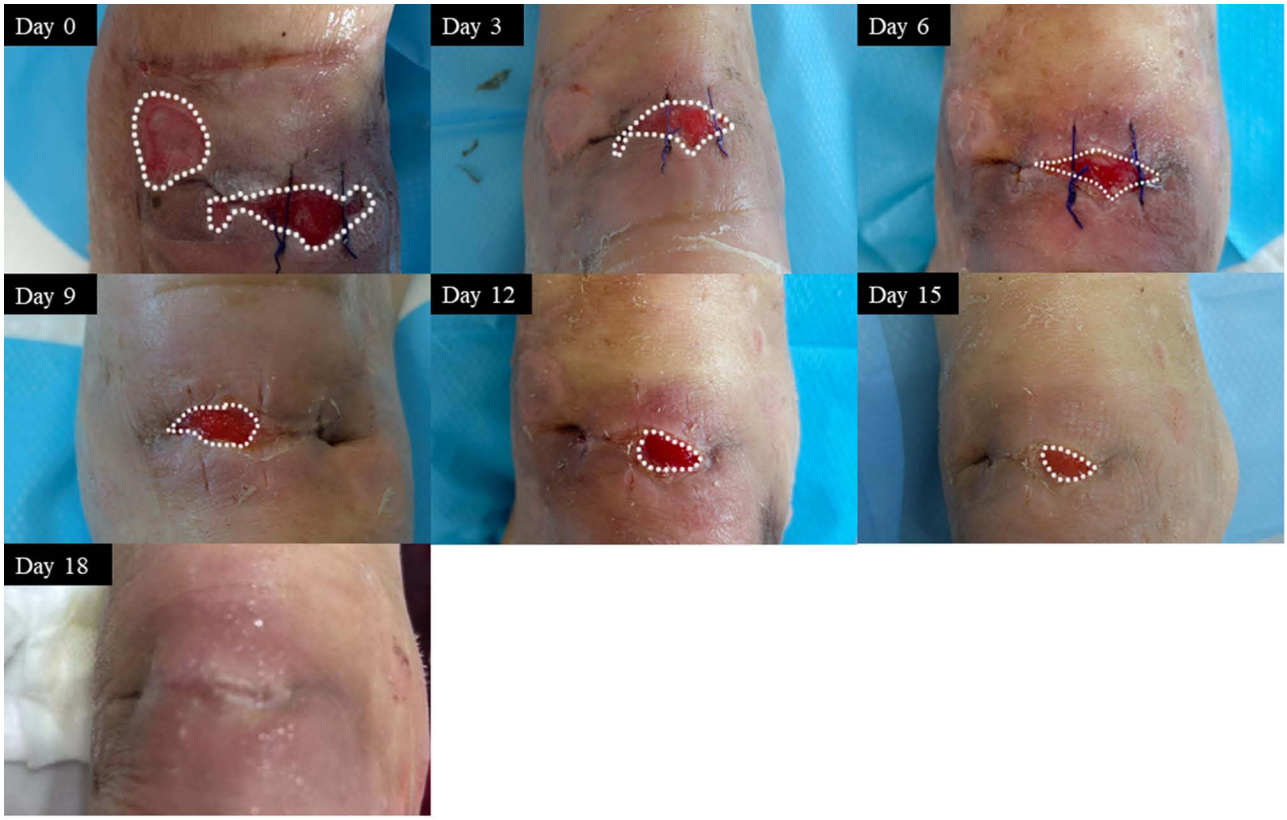

Figure 3 Representative images of the healing process in case 3.

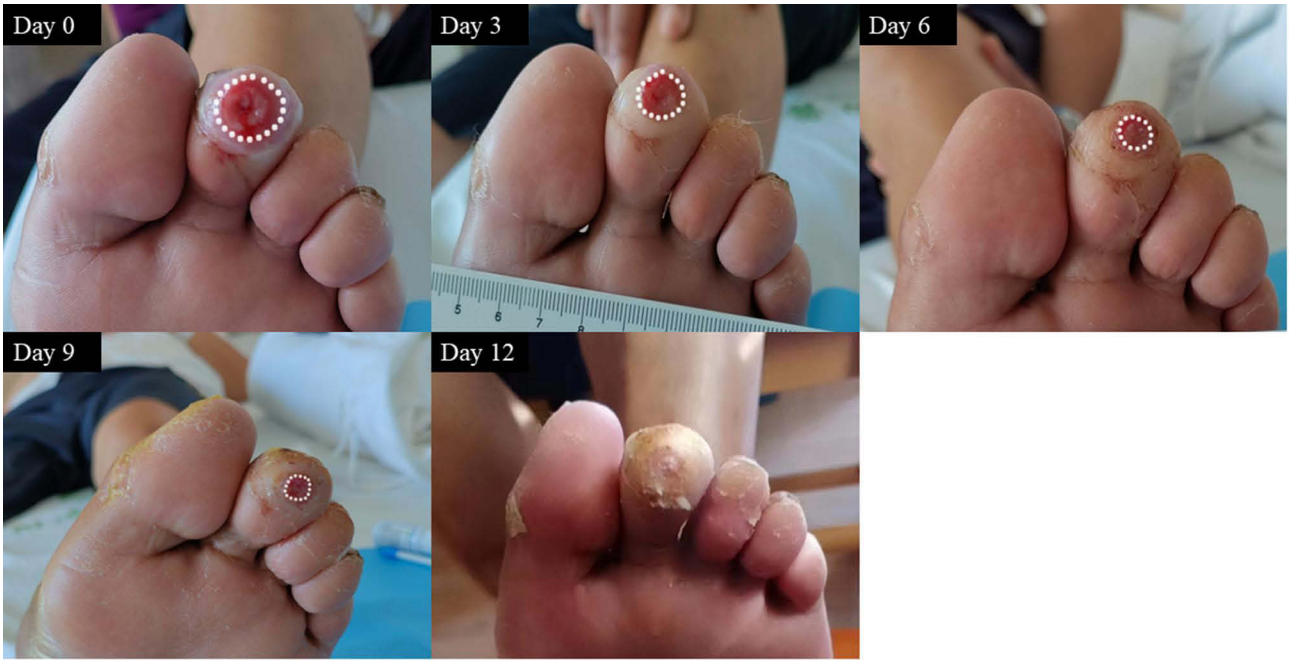

Figure 4 Representative images of the healing process in case 4.

Case 5

A 74-year-old male patient had been diagnosed with T2DM for 16 years who was on miglitol and insulin therapy. He developed an ulcer on the sole of his right foot for 18 days. The ulcer appeared erythematous, swollen, and warm, with other inflammatory manifestations. The patient was treated with conventional dressing therapy and oral antibiotics. The response to the treatment was not good. The patient then presented to our division.

His medical history revealed CKD, atrial fibrillation under anticoagulant therapy, and retinopathy. He was a smoker as well. His weight was 68.9 kg with a BMI 26.3 kg/m^2. On admission, the body temperature of this patient was 36.5°C, with pulse rate of 100 beats/min and blood pressure of 92/62 mmHg. This patient had poor glucose control with HbA1c 10.00%, and ACR was 117.5 μg/mg (Table 1). The size of the ulcer was 2.50 cm^2, with the manifestation of swelling, redness but no pus or discharge. The grade of the ulcer was assessed as Wagner 2, SINBAD 3, and PEDIS 2. Bilateral ankle reflexes were diminished. Vascular Doppler ultrasound of the lower extremities identified thrombosis in the left lower limb. The ABI was within normal range. A microbiological analysis indicated an infection of *Staphylococcus aureus*.

The patient was educated for pressure offloading, and we recommended him to decrease physical activities. An inflatable wedge was used when he was in bed. The patient received antibiotic therapy (Linezolid) for 3 days. After the bacterial culture turned negative, the patient received 4 rhGM-CSF treatments with an initial dose of 5.0 μg along with SOC. Then, the ulcer achieved complete epithelialization (Figure 5).

Case 6

A 69-year-old male patient was admitted to our department presented with a 5-year history of T2DM, who was on metformin and insulin therapy. Over the past 3 years, he has been hospitalized several times for recurrent ulcers on his lower extremities. Four months ago, the patient developed a new-onset ulcer on his right lower limb, accompanied by local skin swelling and purulent secretion exudation. After 3 months SOC including anti-infection and dressing changes in a local hospital, the ulcer did not respond well to these treatments. Then, he was transferred to our division.

He admitted to a history of hypertension, cardiovascular disease, CKD, and smoking. His weight was 71 kg with a BMI 25.20 kg/m^2. On admission, the patient had a fever with a body temperature of 37.5°C, pulse rate of 79 beats/min, and blood pressure of 148/80mmHg. The level of HbA1c was 7.40%, and ACR was 142.5 μg/mg (Table 1). The size of

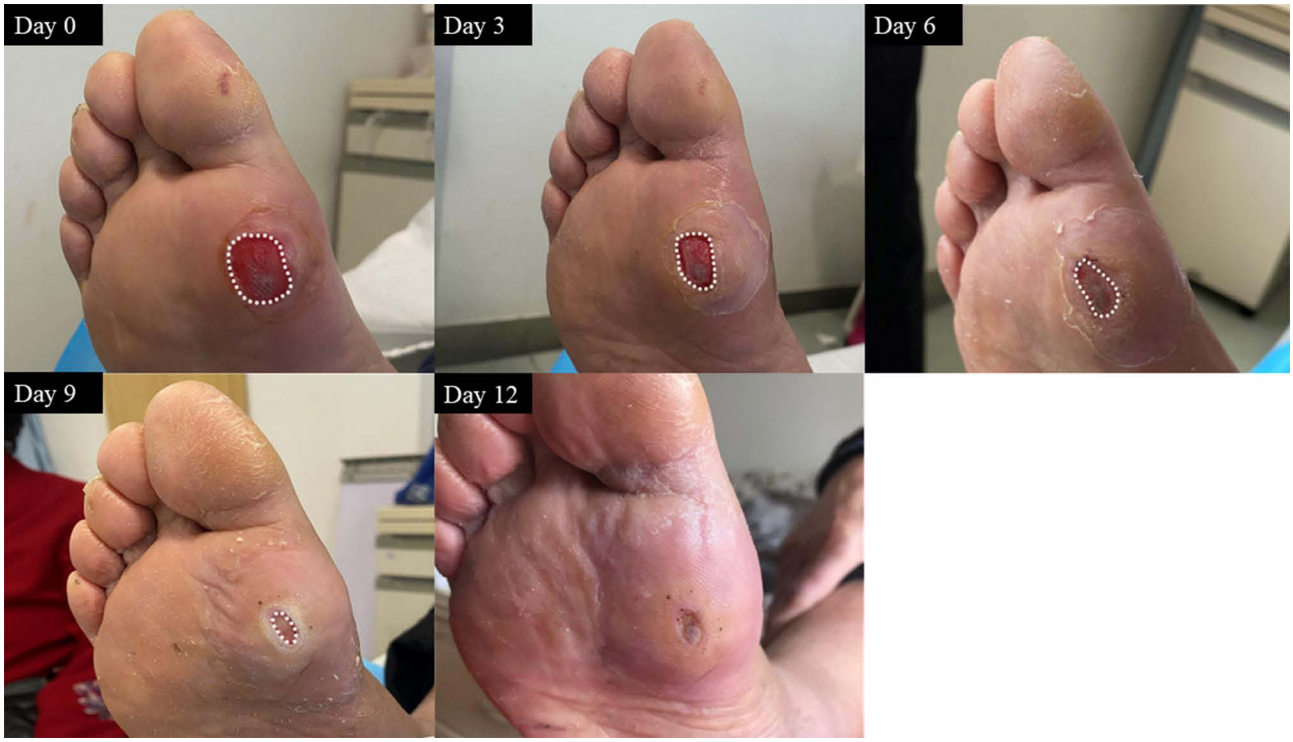

Figure 5 Representative images of the healing process in case 5.

the ulcer was 10.91 cm^2, with purulent exudation over the ulcer. The ulcer evaluation was Wagner 3, SINBAD 3, and PEDIS 3. Bilateral ABI was in the normal range. ESR and hsCRP were sharply elevated, indicating severe infection. *Staphylococcus aureus* was identified in the microbial culture.

After antibiotic therapy (Teicoplanin, Sulbactam/Cefoperazone) for 10 days, the infection was efficiently controlled. And then the patient received 6 rhGM-CSF treatments with an initial dose of 21.82 μg. After that, the ulcer achieved complete epithelialization (Figure 6).

Case 7

A 65-year-old male patient with a 13-year history of T2DM was admitted to our department due to a skin ulcer on the little toe side of his left dorsal foot for 4 weeks. The ulcer manifested as swelling, redness, and pain, accompanied by elevated skin temperature. After 2 weeks SOC including anti-infection and dressing changes in a local hospital, the ulcer did not respond well. In addition, his oral hypoglycemic drugs included metformin and glimepiride but his glucose control was poor.

He had a history of hypertension, cardiovascular disease, CKD, and smoking. His weight was 62 kg with a BMI 22.20 kg/m^2. On admission, the patient had a fever with a body temperature of 37.6°C, pulse rate of 84 beats/min, and blood pressure of 122/65mmHg. The level of HbA1c was 10.30%, and ACR was 9.3μg/mg (Table 1). The size of the ulcer was 6.24 cm^2, and the ulcer evaluation was Wagner 2, SINBAD 3, and PEDIS 2. Bilateral ABI was in the normal range. C*oagulase negative staphylococcus* was identified from the microbial culture along with slightly elevated ESR and hsCRP.

We suggested that this patient reduce his physical activity and an inflatable wedge was applied for offloading. After antibiotic therapy (Ceftriaxone), the patient received 5 rhGM-CSF treatments with an initial dose of 12.48 μg. After that, the ulcer achieved complete epithelialization (Figure 7).

Case 8

A 29-year-old male patient presented with a case of T2DM for 3 years, who was on metformin, saxagliptin, and insulin therapy. The patient developed a new-onset ulcer on his right lower limb one month ago. The ulceration manifested as

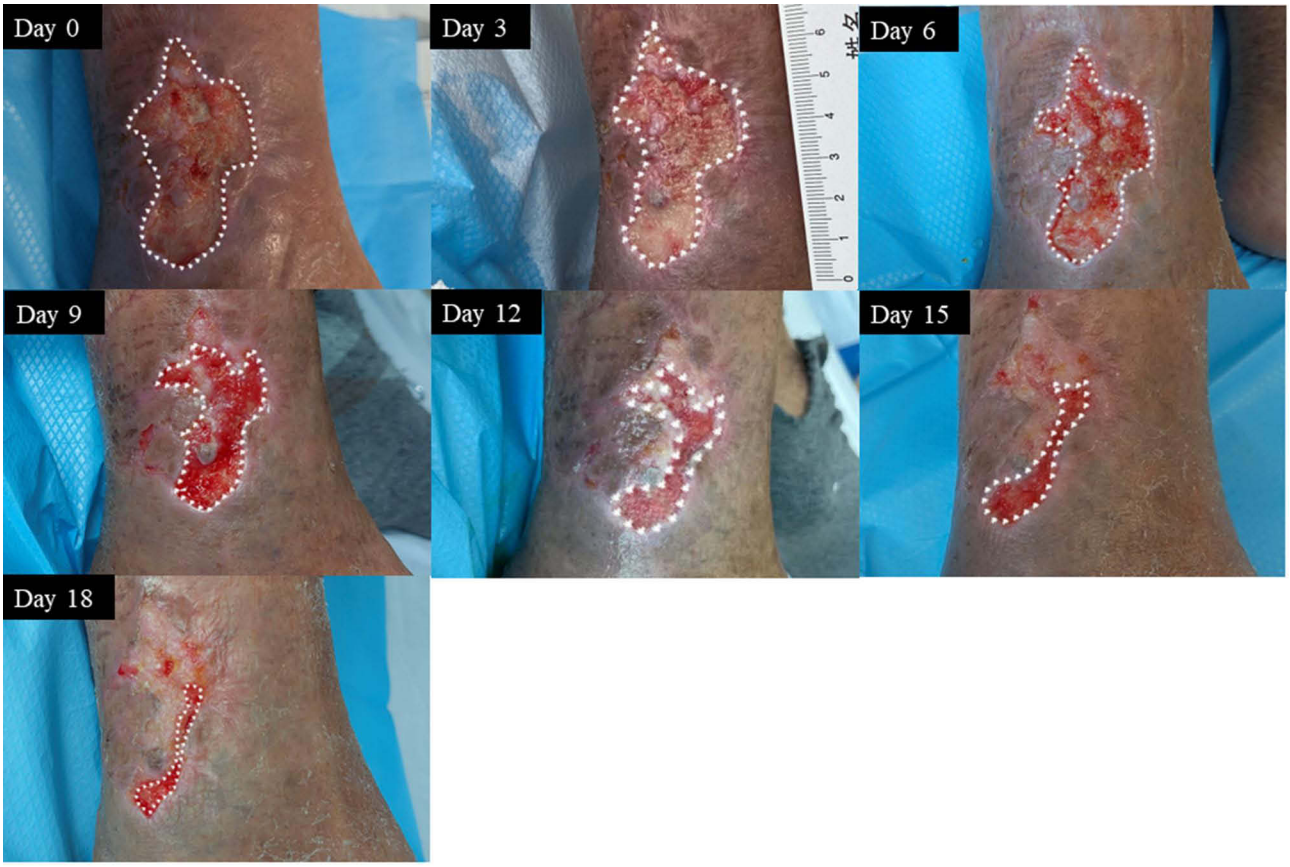

Figure 6 Representative images of the healing process in case 6.

local skin redness, heat, swelling, and purulent secretion. The patient received antibiotic therapy and dressing changes at the surgical clinic of a local hospital but the wound was not healed.

He had a history of anaphylactoid purpura nephritis and smoking. His weight was 64 kg with a BMI 21.10 kg/m^2. On admission, the patient showed a body temperature of 36.6°C, pulse rate of 110 beats/min, and blood pressure of 109/82mmHg. The level of HbA1c was 12.70%, and ACR was 586.6 μg/mg (Table 1). The size of the ulcer was 6.74 cm^2 with purulent exudation over the ulcer. The ulcer evaluation was Wagner 2, SINBAD 3, and PEDIS 3. Bilateral ABI was in the normal range. A microbiological analysis indicated an infection of *Staphylococcus aureus*.

After antibiotic therapy (Teicoplanin) for 7 days, the infection was efficiently controlled. The patient then received rhGM-CSF treatments with the initial dose of 13.48 μg. The ulcer achieved complete epithelialization after 5 times of intervention (Figure 8).

Case 9

An 80-year-old male patient with 20-year T2DM was admitted to our department, who was on metformin, glibenclamide, and insulin therapy. An ulcer developed on the right first toe while the patient trimmed his toenails at home. Then, it turned red and swollen with purulent secretion. The patient was treated with antibiotics and conventional dressing changes in a local hospital. The ulcer failed to complete healing after 6 months of treatment. The patient then presented to our division for further treatment.

His medical history revealed hypertension, CKD, and smoking. The body weight was 75 kg with a BMI 27.5 kg/m^2. On admission, the body temperature was 36.2°C, pulse rate was 80 beats/min, and blood pressure was 168/73mmHg. The level of HbA1c was 6.30%, and ACR was 29.1 μg/mg (Table 1). The ulcer appeared red and swelling without purulent discharge, the size of which was 2.63 cm^2. The grade of ulcer was Wagner 1, SINBAD 3, and PEDIS 2. The left ABI was 0.75, and the right ABI was 0.50. CTA indicated moderate-to-severe stenosis of bilateral lower extremity arteries. In

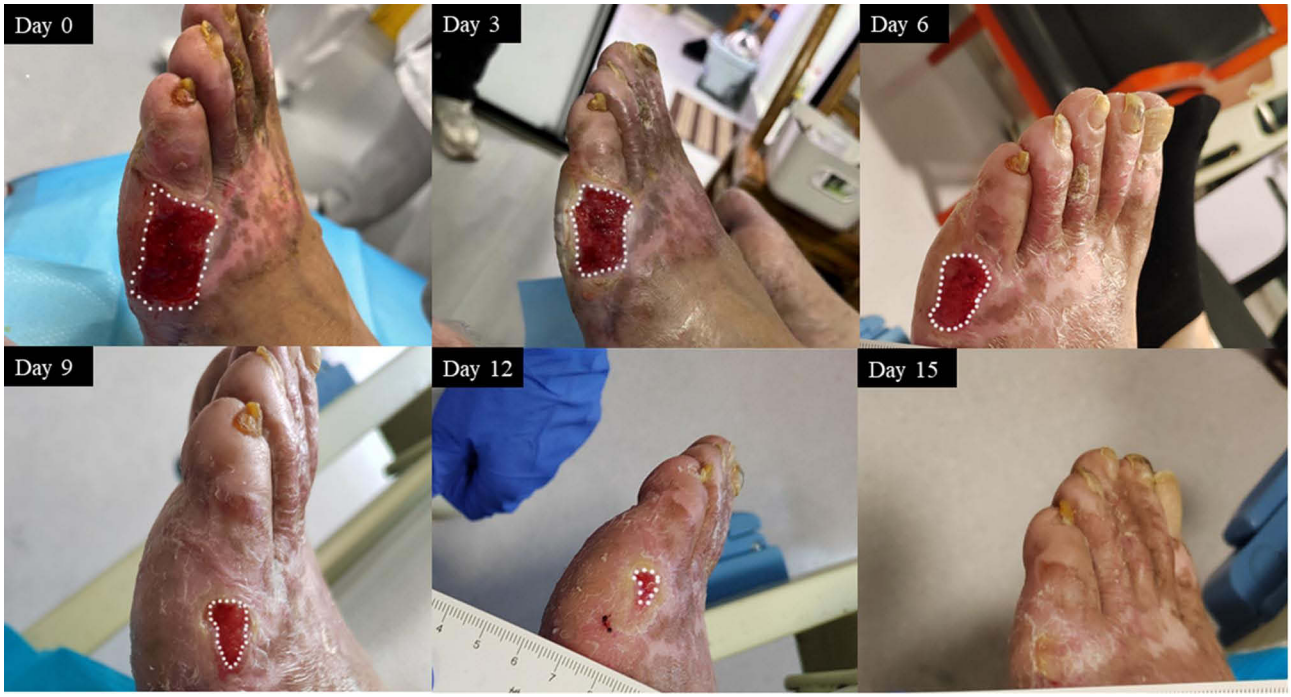

Figure 7 Representative images of the healing process in case 7.

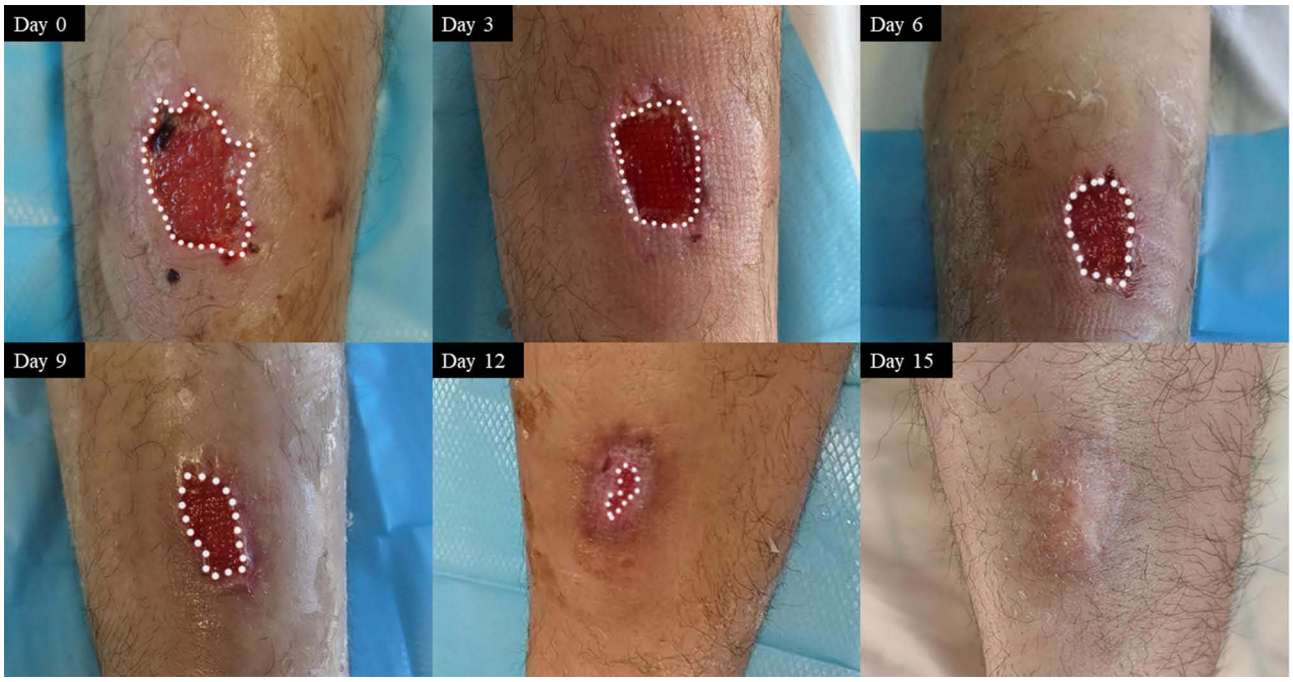

Figure 8 Representative images of the healing process in case 8.

addition, his bipedal sensory function was reduced. A tissue sample from the ulcer was collected, and *Candida parapsilosis* was identified.

The patient received percutaneous balloon angioplasty and antibiotic therapy (Piperacillin Sodium and Tazobactam Sodium). An inflatable cushion was used for pressure offloading. After the bacterial culture turned negative, he received rhGM-CSF treatment with an initial dose of 5.26 μg. After 5 rhGM-CSF treatments along with SOC, the ulcer achieved complete epithelialization (Figure 9).

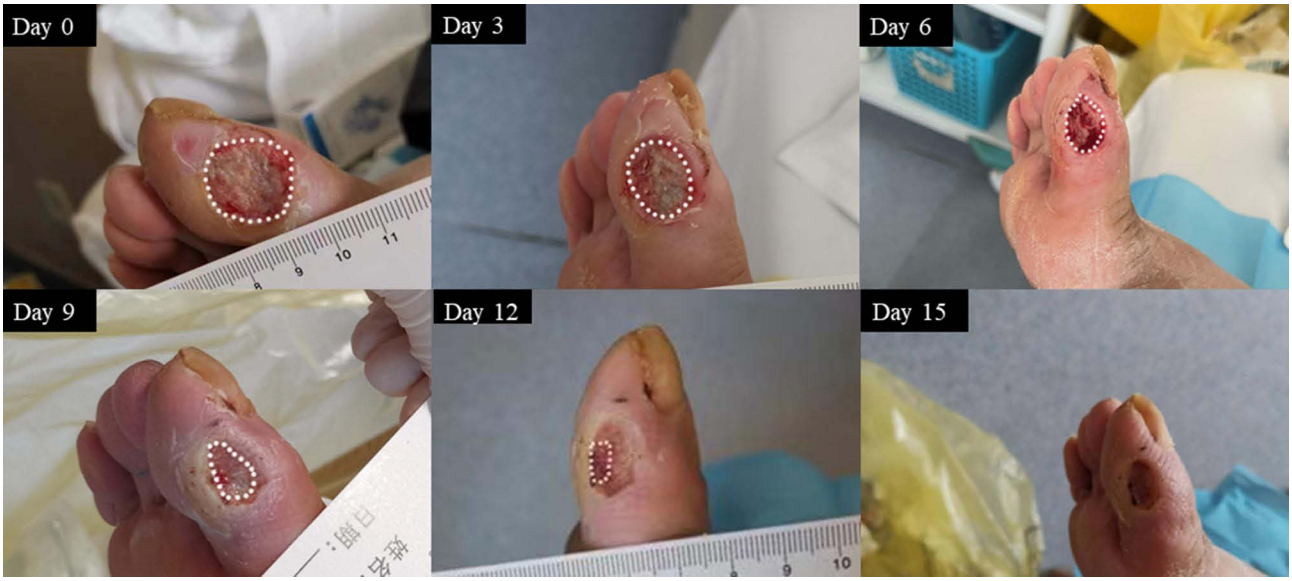

Figure 9 Representative images of the healing process in case 9.

Results

This case series included 9 diabetic patients with lower extremity ulcers aged from 29 to 80 years. The duration of ulcers varied from 14 to 210 days. All the patients were male with one or more comorbidities including retinopathy, peripheral neuropathy, hypertension, cardiovascular disease, and CKD. Six of 9 patients presented poor glucose control with HbA1c more than 7% and only 4 patients had normal ACR (Table 1).

Except for 2 ulcers on the leg, all other ulcers were located on feet. The average area of the included ulcers was 5.3 ± 2.7cm^2. Seven of 9 patients were neuropathic ulcers, and the ABI of the affected limb was 1.03 on average. Only 1 patient's ABI was below the normal reference range. As shown in Table 1, the Wagner grade ranged from 1 to 3, SINBAD grade ranged from 2 to 4, and PEDIS ranged from 2 to 3. The majority of tissue samples (8 of 9) identified positive pathogens, the predominant organism being *Staphylococcus aureus*.

In the current cases, all 9 patients received standard wound treatment including glycemic control, debridement of necrotic tissues, topical wound dressings, antibiotic therapy, and foot care education. All patients were educated about pressure offloading. Inflatable wedge or cushion was used in all cases except for case 6 and 8. In addition, case 3 received VSD treatment and case 9 received percutaneous balloon angioplasty. rhGM-CSF was topically administered on the surface of non-healing wounds (2.0 μg/cm^2) and then covered with sterile wound dressing every 3 days. The area of all ulcers presented a gradually decreasing trend (Figure 10). After 4–8 times of rhGM-CSF treatments, all the ulcers in the present case series achieved complete epithelialization (Figure 10). The mean time to complete healing was 16.0 ± 3.7 days. The relative ulcer area was calculated as a percentage of the initial ulcer area. As shown in Figure 11, the relative ulcer area reduced to 66.7 ± 13.0% on average after the first treatment. Moreover, the healing velocity during the whole observation period was 0.32 ± 013 cm^2/day.

Discussion

Wound healing is a complex, dynamic, multi-cellular biological process, which is achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling.[30] These orderly processes are impaired under the condition of diabetes, mainly attributed to several intrinsic and extrinsic factors, such as neuropathy, blood supply insufficiency, wound infection, and dysfunction of resident cells in diabetic ulcers (eg, fibroblasts, epidermal cells, and macrophages), which contributes to the delayed healing of DFU.[31] Nowadays, increasing attention has been paid to developing efficient and economic adjunctive strategies for the treatment of DFU.

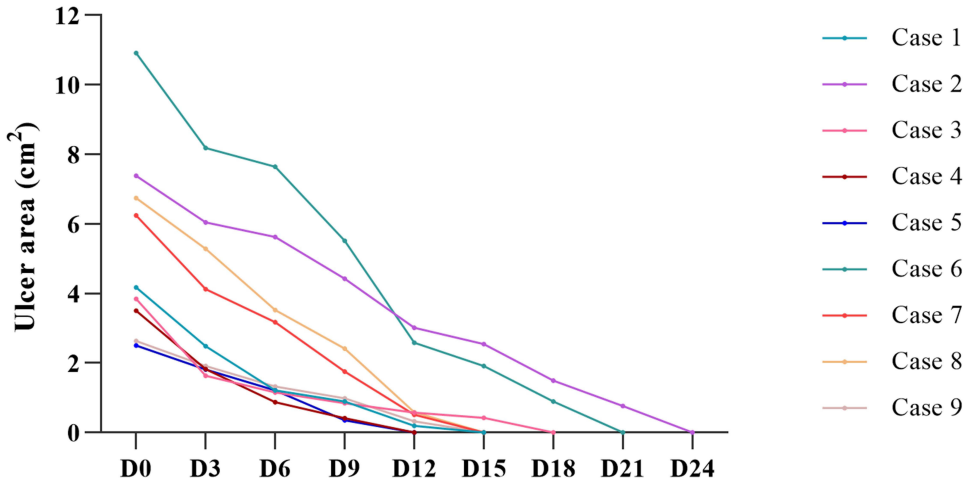

Figure 10 Wound size of all 9 patients during rhGM-CSF treatment.

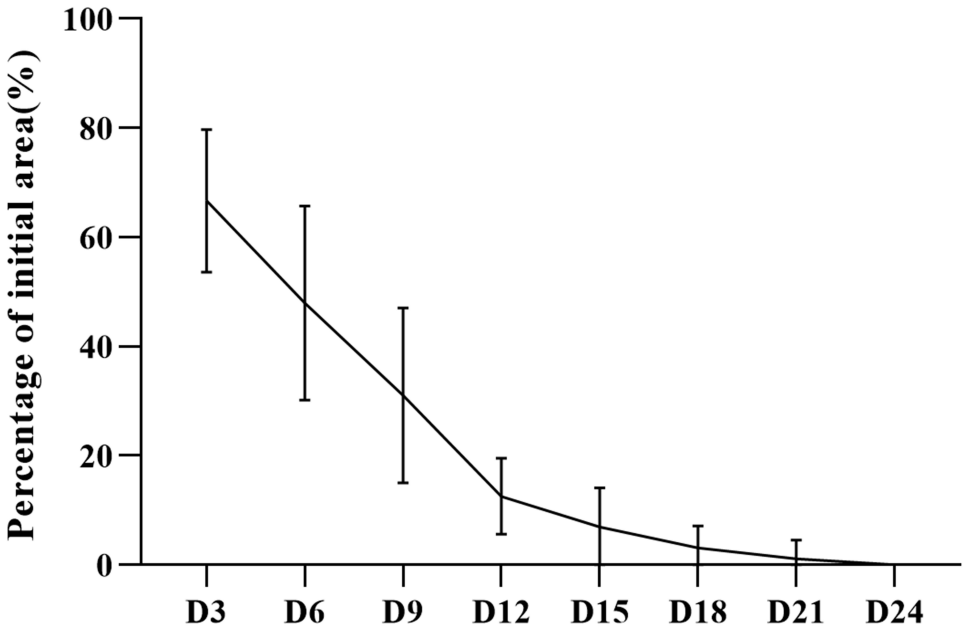

Figure 11 Relative area size during rhGM-CSF treatment. Data were shown as the percentage of initial ulcer area.

Although GM-CSF is traditionally applied in the treatment of blood system diseases, multiple in vitro studies disclose that GM-CSF may play an essential role in the cascade of the healing process due to its function in the modulation of several key resident cells in wounds, such as fibroblasts, keratinocytes, and macrophages.[32–34] Postiglione et al reported the expression of GM-CSF receptor in human fibroblasts. The proliferation activity of fibroblasts was modulated by different concentrations of GM-CSF.[32] In addition, GM-CSF has been confirmed to promote angiogenesis in tumor tissue,[35] mainly through promoting the activation of macrophages, and then releasing VEGF and other vascularizing cytokines.[36] Animal studies further verified the role of GM-CSF in wound repair.[16,17,37–39] Mann et 27227al reported that GM-CSF overexpression in transgenic mice promoted re-epithelization and wound closure by stimulating keratinocyte proliferation, granulation tissue formation, and vascularization.[16] On the other hand, wound healing was significantly delayed in GM-CSF knockout mice.[17] Furthermore, Canturk et al verified the beneficial effects of GM-CSF on incisional wound healing in an experimental diabetic rat model.[38] We also explored the potential mechanisms of GM-CSF in wound healing. Delayed healing of DFU is partly caused by the imbalance of M1/M2-type macrophages.[40] Restoration of the

balance between these macrophage types may promote wound healing. Our unpublished data indicated that GM-CSF intervention could promote the transition of macrophage from M1 to M2, increase the level of VEGF within the ulcer, and accordingly stimulate vascularization (unpublished data).

Furthermore, a multitude of clinical studies demonstrate that GM-CSF has been employed in the treatment of various non-diabetic ulcers including chronic venous ulcers, surgically induced wounds, leg ulcers, and burn wounds over the past decades.[18–20,22,41] A meta-analysis investigating the effect of GM-CSF on deep second-degree burns demonstrated that the use of rhGM-CSF significantly reduced wound healing time by 4.77 days.[41] Jaschke E et al topically applied GM-CSF among 39 patients with chronic venous insufficiency and identified that the rate of healing can be significantly improved with the average healing time 19 weeks.[18]

However, the application of GM-CSF in DFU is rarely reported. The current case series included 9 cases with refractory diabetic ulcers that responded not well to the standard wound treatment. The ulceration duration ranged from 14 to 210 days. Our results showed that the ulceration in all the 9 patients achieved complete epithelialization after 4–8 times of topical rhGM-CSF treatments. Of note, the ulceration of case 2 failed to achieve healing after 7-month SOC. After 8 times of rhGM-CSF intervention, the ulceration completely healed (Figure 10). A cross-sectional study of 2513 patients with chronic cutaneous wounds in China found that the median DFU healing time was 76 days for men and 117 days for women under conventional therapy alone.[5] In the UK, the mean time to healing was 4.4 months with currently available treatments.[42] Even after 1-year treatment, approximately 23% of patients still have unhealed DFUs, according to the data from a prospective cohort study of 1088 DFU patients across 14 centers in Europe. In the current case series, the mean time to complete healing after GM-CSF intervention was 16.0 ± 3.7 days, which markedly reduced the healing time. In addition, the healing velocity of the included patients was 0.32 ± 013 cm^2/day. It has been summarized by our previous review that the healing velocity after the application of endogenous cytokine therapy, that is autologous platelet-rich plasma (PRP), ranged from 0.385 to 0.867 cm^2 /week.[43] Obviously, the intervention of exogenous cytokine GM-CSF is not inferior to PRP. Taken together, our findings disclosed the marked therapeutic effectiveness of rhGM-CSF on diabetic ulcers.

Furthermore, DFUs lead to a substantial economic burden worldwide.[44,45] The per-person incremental annual health care costs for Medicare in the United States were $11,710 in patients with DFUs.[46] The annual health care costs for DFUs in England were £837 million.[47] In the current study, one ampoule of rhGM-CSF (100 μg) costs $7.7, which is equivalent to $ 0.154 per cm^2 of ulcer area. Accordingly, rhGM-CSF intervention could be a cost-effective adjunctive strategy, which may markedly ameliorate the economic burdens of patients with DFUs.

The strength of this study is that it is the first case series investigating the effect of rhGM-CSF on diabetic ulcers. However, this case series was limited due to the sample size. A randomized controlled trial by our research team is in progress, which may further verify the therapeutic effectiveness for diabetic ulcers. In addition, the cost-effectiveness of rhGM-CSF treatment will be analyzed as well.

Conclusions

The present case series involving 9 cases of diabetic ulcers for the first time revealed that rhGM-CSF is effective in promoting ulcer healing. It can be an attractive option as an adjuvant treatment for refractory diabetic ulcers.

Ethics Statement

This study was approved by the Ethics Committee of Tongji Hospital, Huazhong University of Science & Technology (Reference number: TJ-IRB20211212). Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

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Disclosure

The authors declare no conflicts of interest in this work.

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