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High Matrix Metalloproteinase Levels Are Associated With Dermal Graft Failure in Diabetic Foot Ulcers

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Abstract

The aim of our study is to analyze factors, including matrix metalloproteinase (MMP) levels, that could influence the integration of dermal grafts in diabetic foot ulcers. From September 2012 to September 2013, 35 diabetic patients with IIA lesion (Texas Wound Classification) and an extensive foot tissue loss were considered suitable for dermal graft. Before the enrollment we ensured the best local conditions: adequate blood supply, control of infection, and offloading. The MMP level of each lesion was evaluated blindly before the application of dermal substitutes. At 1-month follow-up, we analyzed the correlation between clinical patient characteristics, local wound features including MMP levels, dermal substitute applied, and the outcome expressed in terms of dermal graft integration. We observed dermal graft integration in 28/35 patients (80% of our population). In multivariate analysis high MMP level was the only negative predictor for dermal graft integration ($P < .0007$). In addition, we divided the patients into 2 groups according to MMP levels: group 1 with low protease activity (24 patients) and group 2 with elevated protease activity (11 patients). The integration of the dermal graft was 100% in group 1 (n = 24 patients) and 36.4% in group 2 (n = 4patients), $P < .0001$. According to our data, the evaluation of MMP levels may be useful to choose the right strategy to get the best results in terms of clinical success and cost saving. However, further studies are necessary to confirm these findings.

Keywords

diabetic foot, ulcers, matrix metalloproteinases, acellular dermis

Diabetic foot ulceration is a serious complication of diabetes mellitus and the most common cause of hospitalization in diabetic patients.[1] The risk of a diabetic person to develop a foot ulceration during his life is 15%.[2] Diabetic foot ulcers are the most costly type of chronic wounds to care for. It poses as a considerable problem in health care.

Clinicians' understanding and ability to achieve wound healing in diabetic foot ulcers has increased significantly over the past years. Recently, a new therapeutic perspective has been represented by "regenerative surgery" with the use of tissue-engineered products.[3,4] Among these, dermal substitutes are scaffolds composed of extracellular matrix (ECM) components implanted in full-thickness skin wounds to reconstruct the dermis.[5,6] Dermal grafts supply a structural and functional scaffold regulating the proliferation and differentiation of keratinocytes and inducing the formation of a functional basement membrane.[7]

However, they are expensive and their integration in the wound bed is not always achieved because of several systemic and local factors that can interfere with their integration.

A physiological wound healing needs the following conditions: adequate blood perfusion, the absence of any traumatic force or pressure on the ulcer area, absence of infection, good nutritional, and general health status.

Several studies analyzed wound bed conditions describing the differences between healing and nonhealing wounds, highlighting the role of matrix metalloproteinases (MMPs).[8-12] MMPs are enzymes that act on proteins, cutting up their protein molecule. Components of ECM such as collagen, gelatin, and proteoglycans are substrates of MMPs. Elevated levels of MMPs and reduced levels of their endogenous tissue inhibitors have been shown in nonhealing wounds.[10-12] It has been hypothesized that increased activity of these enzymes could cause excessive degradation of ECM proteins, interfering in a key passage of wound healing.[13,14]

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The aim of our study is to analyze factors, including MMP levels, that could influence the integration of dermal grafts in diabetic foot wounds apparently free from any interfering condition that could impair the healing process.

Research Design and Methods

From September 2012 to September 2013, all the diabetic patients presenting at the Department of Internal Medicine, U.O. Diabetic Foot, University of Tor Vergata Rome, with an A2 lesion according to the Texas Wound Classification (TWC) and a foot tissue loss related to an aggressive debridement due to previous infection or gangrene, were considered suitable for dermal graft. According to our standard protocol, we ensured the best local conditions available to allow the wound healing: control of infection by aggressive debridement and antibiotic therapy (initially broad spectrum empirical therapy and then microbiological analysis), adequate local blood supply by peripheral revascularization, and reduction of high plantar pressures by offloading.

The wound was considered suitable for dermal substitute in case of

- Absence of clinical signs of infection (absence of purulent secretion, pain, edema, cellulitis)
- Adequate blood flow established by $TcPO_2 > 30$ mm Hg
- Absence of necrotic tissues

Each patient was assessed for sex, age, type and duration of diabetes, glycated hemoglobin (A1c) levels, serum creatinine levels, nutritional values (serum albumin, pre-albumin, retinol binding protein, transferrin, lipids), and previous lower limb revascularization. Local blood perfusion and peripheral neuropathy were evaluated by percutaneous oximetry ($TcPO_2$) and vibration perception threshold (VPT), respectively.

We analyzed the correlation between clinical baseline conditions (sex, age, type and duration of diabetes, A1c levels, serum creatinine, nutritional values), foot sensitivity (VPT), local blood perfusion ($TcPO_2$ value), wound bed features (ulcer area, MMP levels), dermal substitute applied, and the outcomes expressed in terms of dermal graft integration.

Protease Test

To evaluate MMP levels, each lesion was assessed blindly before the application of the substitute.

We used the WOUNDCHEK Protease Status (Systagenix), a rapid diagnostic test developed to evaluate MMP levels in chronic wounds, which provides results in 15 minutes. It is an immunochromatographic test for the qualitative assessment of human neutrophil–derived inflammatory protease activity.

The test consists of different steps:

1. Put test card flat on the work surface and add 4 drops of reagent solution to the top hole.
2. Clean the wound with sterile saline and ensure that complete hemostasis has been achieved.
3. Roll the swab flat against the wound bed until the complete surface is coated by the wound fluid.
4. Insert the swab into the bottom hole of the test card and rotate it 5 times.
5. After 10 minutes, close the card, removing the adhesive liner from the right edge, and wait for 5 more minutes.
6. Read the test results. If the Control line (C) does not appear the test is not valid. If the Control line appears, compare the color intensity of the Test line (T) to the color intensity of the Result Interpretation Reference Line (R), on the strips provided in the test.
    - If T line is lighter than R line, or T line is not visible at all, the sample contains elevated levels of MMPs.
    - If T line is darker than or equal to the color of R line, the sample contains low levels of MMPs.

Implantation of Dermal Substitutes and Follow-Up

After the surgical debridement, patients underwent the dermal grafting. The clinician who performed the dermal graft was not aware of the MMP levels of the lesion.

We applied 2 different types of dermal substitute, based on the availability in the hospital:

- Integra dermal regeneration template is a 2.1-mm thick bi-laminar skin substitute composed of a layer of bovine tendon collagen type I matrix and shark chondroitine-6-sulfate and a silicone layer that acts as a temporary pseudo-epidermis (Figure 1)
- Hyalomatrix is a synthetic acellular dermal analog of 2-mm thickness composed of esterified hyaluronic acid fiber matrix beneath a silicone membrane

The grafted area was covered with nonadherent paraffin gauzes and a secondary dressing made of sterile gauzes. The secondary bandage could be changed every 5 days, but the nonadherent gauzes had to be left until our control visit to be changed by the clinician. All patients had homogeneous removable offloading.

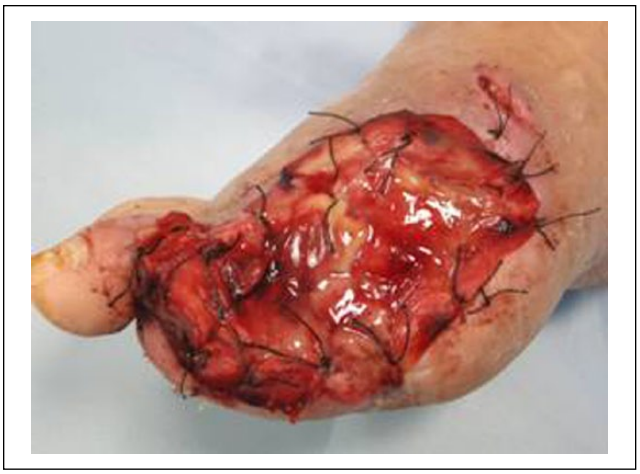

Figure 1. Integra application.

The first control visit was carried out after 2 days. The follow-up was scheduled at 7, 14, 21, and 30 days.

At 1-month follow-up, the dermal graft was considered successful if there was no plane of cleavage between the wound bed and the dermal substitute; it was considered as failure if the dermal replacement was still separated from the wound bed and easily removable. Also, the clinician who evaluated the graft integration was not aware of the MMP levels recorded when the graft was applied.

Statistical Analysis

Statistical analysis was performed using SAS (release 8; SAS Institute, Cary, NC) for personal computers. Data are expressed as means ± standard deviation (SD). Comparisons between groups were made with χ^2 test (frequency data) or ANOVA (continuous data). Univariable logistic regression analyses were performed for all potential predictor variables with the outcome of interest, with values presented as odds ratio (OR) along with the respective 95% confidence interval (CI). All potential predictors were entered simultaneously in a multivariable regression and a set of variables that best predict outcome was identified. $P < .05$ was considered statistically significant.

Results

A total of 35 diabetic patients (24 males and 11 females) with lesions ≥2 cm^2 in stage A (no ischemia, no infection) and grade II (wound penetrating the tendon) of the TWC were enrolled. The clinical characteristics of the patients are reported in Table 1.

In total, dermal graft integration was reached in 28/35 patients (80% of our population). No differences were found according to the dermal substitute applied.

In monovariate analysis, we found that duration of diabetes (OR = 2.27; 95% CI = 1.5-4.6; $P = .0246$), serum creatinine (OR = 2.68; 95% CI = 1.06-5.13; $P = .0085$), and high MMP levels ($\chi^2 < .0001$) were significantly associated with the graft failure.

In multivariate analysis, high MMP levels were the only predictor of graft failure ($P < .0007$).

In addition, we divided the patients into 2 groups, according to the MMP levels: 24 patients (group 1) with low protease activity (LPA) and 11 patients (group 2) with elevated protease activity (EPA). A comparison between group 1 and group 2 found no significant differences between all the clinical and biochemical variables, but the serum creatinine level was higher in group 2 than in group 1 (Table 1).

There was a homogeneous distribution of dermal grafts between groups. In fact, we applied 24 dermal grafts in patients of group 1 (13 Integra Double Layer and 11 Hyalomatrix) and 11 grafts in patients of group 2 (6 Integra Double Layer and 5 Hyalomatrix).

The dermal graft integration was observed in all patients of group 1 (100%) and in 4/11 patients (36.4%) of group 2 ($P < .0001$), as shown in Figure 2.

Serum creatinine of patients of group 2 was not different between those with graft integration and those who did not achieve dermal graft integration (3.2 ± 0.99 vs 2.4 ± 1.2).

Discussion

Medical knowledge to achieve wound healing has increased significantly over the past years. The recognition of the role of the ECM in the wound healing process has led to the introduction of products that replace the ECM structure. Among these, dermal substitutes play a key role.[5,6]

In our experience, dermal grafts represent an optimal solution to treat diabetic lesions with an extensive tissue loss. However, in relation to their high cost, it is necessary to ensure adequate wound bed conditions before their application.

Several studies show strong evidence that elevated levels of MMPs prevent wound healing.[14-16] In fact, high MMP levels also degrade proteins that are not their physiological substrate: growth factors, receptors, and ECM proteins.[13,14] Excessive degradation of the ECM could deprive the cells both of the attachment sites and signals required for migration, differentiation, and proliferation.[17]

In diabetic foot ulcer, it is recognized that high levels of MMP-9 degrade ECM, growth factors, and integrins with their respective receptors.[18-21]

Therefore, caution should be exercised before applying dermal substitutes in wounds with EPA because degradation of the matrix is likely to occur.

Table 1. Baseline Characteristics of the Population.[a]

	Total (N = 35)	Group 1 (n = 24)	Group 2 (n = 11)	*P* Value
n (%)	35	24 (68.57)	11 (31.34)	ns
Sex (% male)	68.75	66.67	72.72	ns
Age (years)	66.62 ± 12.5	66.04 ± 13.01	67.9 ± 11.6	ns
Type 2 diabetes (%)	91.43	91.67	90.90	ns
Diabetes duration (years)	18.64 ± 12.8	17.8 ± 12.4	20.25 ± 14.4	ns
HbA1c (%)	8.77 ± 3.2	8.3 ± 2.8	9.94 ± 4.42	ns
Serum creatinine (mg/dL)	1.9 ± 1.5	1.5 ± 0.8	2.94 ± 2.36	.0395
Total cholesterol (mg/dL)	105.2 ± 34.7	111 ± 8	91.8 ± 12	ns
HDL cholesterol (mg/dL)	25.2 ± 9.6	26.4 ± 2.3	22.6 ± 3.4	ns
LDL cholesterol (mg/dL)	52.7 ± 28	57.8 ± 16	49.8 ± 10	ns
Triglycerides (mg/dL)	122 ± 60	123 ± 14	118 ± 21	ns
Serum albumin (g/dL)	2.6 ± 0.6	2.7 ± 0.12	2.4 ± 0.17	ns
Prealbumin (mg/dL)	12.5 ± 6.47	13.6 ± 1.8	10.4 ± 2.4	ns
Retinol binding protein (mg/dL)	4.6 ± 2.9	3.8 ± 0.7	6 ± 3.9	ns
Transferrin (mg/dL)	145.2 ± 2.8	155 ± 12	126 ± 17	ns
Ulcer dimension >5 cm^2 (%)	62.85	66.67	60.67	ns
$TcPO_2$ value (mm Hg)	51 ± 15	49.6 ± 16.2	56.16 ± 12.88	ns
Peripheral angioplasty (yes)	74.28	70.83	81.81	ns
Integra Double Layer (n)	19	13	6	ns
Hyalomatrix PA (n)	16	11	5	ns

Abbreviations: ns, not significant; HDL, high-density cholesterol; LDL, low-density cholesterol.
[a]Data are presented as mean ± standard deviation, unless noted otherwise.

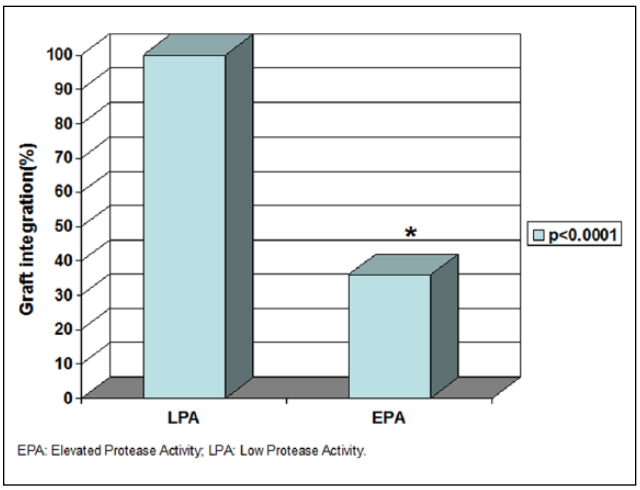

Figure 2. Dermal graft integration according to MMP levels.

According to the recent studies that highlighted the role of MMPs and their inhibitors in the process of wound healing, the evaluation of the wound bed became a key issue in the local treatment of diabetic and nondiabetic ulcers. Several advanced techniques (eg, gelatin zymography, ELISA, fluorimetric assays) are used to analyze levels, types, and activities of MMPs in wound fluid derived by wound biopsy. However, in several cases, laboratory evaluation of MMP levels is not feasible. Furthermore, there are no clinical signs related to EPA. Therefore, it is clearly important to have a rapid diagnostic test to detect ulcers with EPA.

Our experience shows that even in the presence of adequate local conditions (no infection, no ischemia, adequate offloading, good granulation tissue) there were some dermal grafts (20%) that did not integrate in the wound bed. This treatment failure leads also to an economic impact because of the high cost of dermal grafts and the lengthening of the treatment period.

In searching for factors that could predict it, we have found that high MMP levels are related to the failure. In fact, graft failure was observed only in the group with EPA.

The selected patients had no ischemic and no infected lesions when dermal graft was performed. No antibiotic therapy was given. Some previous ischemic patients were included in the study only after successful peripheral revascularization. In fact, $TcPO_2$ values had a mean value of 51 ± 15 mm Hg and no differences were recorded between the 2 groups.

It could be argued that the group with EPA has a significantly higher creatinine level than the group with LPA and that clinical conditions related to renal failure might negatively affect the graft integration. However, analyzing the creatinine inside this group, it comes out that there is no difference in creatinine values between patients with dermal graft integration and those without. In addition,

Table 2. Nutritional Values of Patients in Group 2, According to Graft Integration.[a]

	Graft Integration (4/11)	Graft Failure (7/11)	*P* Value
Total cholesterol (mg/dL)	97 ± 15	88 ± 12	ns
HDL cholesterol (mg/dL)	22.5 ± 2.3	22.8 ± 3.7	ns
LDL cholesterol (mg/dL)	42 ± 9	43 ± 6	ns
Triglycerides (mg/dL)	121 ± 31	111 ± 16	ns
Retinol binding protein (mg/dL)	4.5 ± 2.9	5.6 ± 2	ns
Prealbumin (mg/dL)	12.6 ± 6.8	11.2 ± 4.8	ns
Serum albumin (g/dL)	2.9 ± 0.2	2.8 ± 0.7	ns
Transferrin (mg/dL)	128 ± 11	125 ± 8	ns

Abbreviations: ns, not significant; HDL, high-density cholesterol; LDL, low-density cholesterol.
[a]Data are presented as mean ± standard deviation, unless noted otherwise.

in multivariate analysis, using a model that also includes creatinine, MMP level is the only factor that may predict the graft failure.

Although the nutritional values showed a condition of malnutrition in all the cases, we did not find any significant differences between the 2 groups and among patients with EPA that could lead to implications on graft failure (Table 2).

We have no reasonable interpretation to explain why apparently similar patients and lesions had significant differences in MMP levels. Probably the clinical judgment that has driven our therapeutic choice may take advantage by tests like the WOUNDCHEK Protease Status, with its additional information about MMP levels, which were not available until now. Further studies are necessary to highlight this point.

In conclusion, we state that because of key role of wound bed conditions in the wound healing process, the analysis of MMP levels should be performed mainly when costly therapeutic options such as dermal graft need to be taken.

Declaration of Conflicting Interests

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