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The pioneer use of a modified PRGF–Endoret® technique for wound healing in a hemodialyzed diabetic patient in a terminal stage of renal disease

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Abstract

In the literature, this paper is the first to describe the use of plasma rich in growth factors (PRGF)–Endoret® in hemodialyzed diabetic patients, to promote the healing of after amputation wounds. The PRGF–Endoret® was primarily conceived to be used in maxillofacial surgery, oral implantology, etc., the innovation residing in the blood collection technique (quantity, moment of the week, rhythmicity), which was adapted to the specific conditions of the hemodialyzed patient. Moreover, in the initial phases, the two PRGF fractions were innovatively applied as single alternating layers on the wound surface. Only after the surface of the wound decreased, the two PRGF fractions were applied as overlapping layers. Nevertheless, the paper presents the optimal method to assess the clinical evolution of the wound. Histopathological examination of the biopsy performed during wound preparation for PRGF application brought additional, essential data for orienting the therapeutic approach. The exclusion of calciphylaxis, a disease with high mortality risk, encouraged the application of this method, and also demonstrated the microscopic features in hemodialyzed diabetic patients.

Keywords: PRGF–Endoret®, hemodialysis, diabetes, after amputation wound, calciphylaxis.

Introduction

The uremic patients develop a significant vascular pathology, related to atherogenic conditions such as: hyperparathyroidism (HPTH), dyslipidemia, uremic toxins, amyloidosis [1]. Two different mechanisms explain these vascular complications in end-stage renal disease (ESRD): arteriosclerosis and atherosclerosis. Arteriosclerosis [2] is characterized by hardening of the walls of entire arterial system, associated with thickening and loss of elasticity; the blood flow to the tissues and organs is gradually diminished, especially when combined with atherosclerosis, thus increasing the risk for severe health damages [2]. Atherosclerosis affecting medium-sized vessels is characterized by occlusive lesions due to: thrombogenesis, lipid disturbances, and release of vasoactive substances, growth factors (GFs) and inflammatory mediators. In hemodialysis, the vascular calcifications are frequent in relation with a variety of factors: use of calcium–phosphate binders, parathormone (PTH) over suppression [3], after inflammatory reduction of fetuin-A expression (protein overexpressed in the arterial wall of uremic patients and involved in bone formation) [4, 5], use of Warfarin [which interferes with matrix Gla proteins (MGP), causing osteoporosis] and genetic disturbances [related to the expression of different MGP – fetuin-A, osteopontin, osteoprotegerin and nucleotide pyrophosphatase (NPPS) genes] [6]. Patients with ESRD in a dialysis program have an increased risk (14.4%) of ischemic lower limb ulceration [7]. The prevalence of foot amputation in these patients is about 5.9% [8]. Globally, about 15% of the 150 million diabetic patients exhibit foot ulcerations that evolve into no healing wounds [9]. The impact on the European health budget is huge, almost of 2% [10].

Approximately 50% of chronic wound treatment proved to be effective, and the costs of the therapy are high due to the need for repetitive interventions [11]. In case of chronic skin ulcers, after ischemic amputation, the use of topical solutions for maintaining the wound uninfected seems to be ineffective, since these substances are unable to preserve the wound clean and to accelerate the healing process. Although medical care and nutrition have greatly progressed, novel procedures that are less invasive and more cost effective are still required, to promote wound healing. Platelets play essential roles in hemostasis and wound healing, by secreting a wide variety of cytokines and GFs [12].

Platelet-rich plasma (PRP) is a system for concentrating platelet and plasma proteins. Autologous proteins are obtained from the patient's own blood, shortly before its therapeutic use [13]. PRP application accelerates the repair and regeneration mechanisms in various tissues. At present time, there are different PRP preparation methods (*e.g.*, inclusion of blood cells, such as leukocytes or erythrocytes, using different centrifugation procedures) [9, 14, 15], but the only one approved by American *Food and Drug Administration* (FDA) is plasma rich in GFs (PRGF). PRGF contains more than 350 000 platelets/μL, and no leukocytes. It is prepared from 5–80 cm^3 of blood, taken from a peripheral vein and collected in the presence of sodium citrate. The blood is centrifuged for eight minutes at a specific speed (460×g). Using this technique, the leukocytes concentrate in the plasma immediately above the red cells, in a single layer. There are two fractions of plasma formed right on top of white cells: the fraction with a lower amount of GFs but rich in fibrin is the upper 60%, and below this, the remaining 40% corresponds to PRGF [9], that is easy to collect, without leukocytes. The secretion of GFs begins with platelet activation. Hence, PRGF technique uses calcium chloride solution. To optimize the process, calcium concentration must be determined. The PRGF protocol separates the plasma into two fractions: fraction 1 contains the similar number of platelets as the peripheral blood and is used to provide the fibrin network; fraction 2 contains the highest platelets content [16] and it is very reach in GFs. The use of an autologous fibrin matrix and biologically active GFs locally applied to the damaged skin areas facilitates cell renewal and improves the healing process in a more natural way.

We used PRGF–Endoret®, the PRP–PRGF system developed by the Biotechnology Institute (BTI – a Spanish multinational company specializing in biomedicine and biotechnology). This product is certified by the health authorities in Europe and approved for obtaining and applying PRP in oral and maxillofacial surgery, oral implantology and in other medical fields [17]. Endoret® stimulates tissue regeneration due to its content in GFs in higher concentrations than those of blood [17].

PRGF–Endoret® has proven to be effective [17] in promoting wound healing in general, but the technique has not been applied to dialysis patients. This fact was due partially to the anemia inherent in patients with chronic renal failure, especially in those undergoing hemodialysis, which is associated with additional loss of blood. Uremic toxins as well as the proinflammatory status encountered in these patients are other impediments in using PRGF. Moreover, in the diabetic patient, blood glucose is a good culture medium for bacteria. Heparin used as an anticoagulant in the extracorporeal circuit may interfere with blood clot formation in the process of obtaining the PRGF.

Aim

Considering not only the complex vascular pathology and other associated conditions in the diabetic patients undergoing hemodialysis, but also the high incidence of the chronic skin lesions that occur in these patients, our study aimed at demonstrating the beneficial effect of a pioneer modified PRGF–Endoret® procedure in after-amputation wound healing and the importance of implementing this new, noninvasive, and economic technique.

Patient, Materials and Methods

The study was performed on a 65-year-old female patient, enrolled, for the last nine years, in a high-flux hemodialysis program. She was diagnosed, 25 years ago, with type 2 diabetes mellitus and diabetic nephropathy; she also presented high values of hypertension, dyslipidemia, tertiary HPTH and angiographically documented obliterate chronic arteriopathy (yet, with no requirements of vascular procedures). Under these circumstances, 18 months in advance, an ischemic lesion occurred at the first and second toe of the right foot, with a proximal extension, with the subsequent infection of the wound. To preserve the healthy tissue as much as possible, an amputation of the distal part of the right foot was performed. Seven months later, despite the classical surgical methods of bandaging, the surgical wound did not heal, a big area of necrotic tissue being still present at the amputation site. The removal of the necrotic tissues was performed and a biopsy was collected in order to establish the type and the extent of the vascular injuries, as well as the presence of calciphylaxis (possible due to HPTH), that could also explain the delayed wound healing.

After the necrectomy (Figure 1), the large surface of the wound required a rigorous monitoring for clinical parameters (blood pressure, cardiac rhythm, temperature, arterial–venous fistula examination) and paraclinical parameters [kT/V – dialysis efficiency, hemoglobin (Hb) – anemia status, PTH/calcium/phosphate – calcium–phosphorus metabolism, C-reactive protein (CRP) – pro-inflammatory status, glycated Hb (HbA1c) – long-term control of diabetes mellitus, glycemia]. The imbalances of calcium–phosphorus metabolism were treated with a regular daily dose of 4.8 g Sevelamer carbonate (a phosphate binder that was administered during all hospitalization interval), while for normalizing the high values of glycemia, insulin was administrated. During the entire hospitalization period, to prevent the contamination of the wound, an antibiotic treatment strategy was initiated, according to the antibiogram. Before PRGF therapy, the patient had an infection with *Enterococcus*, which was treated with Amoxicillin 3×500 mg/day for 10 days; after PRGF therapy, in the fifth month of re-epithelization, the patient had an infection with *Staphylococcus aureus*, which was treated with Vancomycin adjusted to the renal dysfunction 1 g/week for two weeks.

The PRGF treatment was initiated according to an innovative protocol, consisting of several steps with weekly application, on the day after the third hemodialysis session of the week (Table 1).

After preparation, PRGF was applied weekly (Figure 2), on the consecutive day of the last hemodialysis session of the week. Four days after applying PRGF, the sterile bandage was removed, the wound was cleaned with a sterile normal saline solution and the wound was covered

again with sterile bandages and intermittently with silver bandages. Cultures from the surface of the wound were commonly collected twice a week, followed by a bacteriological examination was performed. Complete blood count (CBC) and inflammatory biomarkers were assessed twice a month.

Table 1 – ***The steps of PRGF–Endoret® modified protocol***

Prelevation of 36 mL venous blood in eight vacutainers with sodium citrate.
Blood centrifugation.
Separation in sterile conditions of the plasma fractions 1 and 2.
Clotting activation using calcium chloride solution.
Splitting the plasma fractions in 2–3 recipients each, depending on the wound size.
After 20 minutes: tissue application – firstly fraction 2, then above, fraction 1 (Figure 2); if fraction 2 was not enough to cover the entire surface of the wound, it was applied in a single layer on the necrotic areas (medially), whereas fraction 1 was layered in the proximity, in the less damaged zones of the wound (laterally).
Covering with sterile cotton dressing for four days.
Local wound evaluation (removal of the excessive fibrin and cellular debris, flushing with serum saline).
Re-covering with sterile cotton dressing.

PRGF: Plasma rich in growth factors.

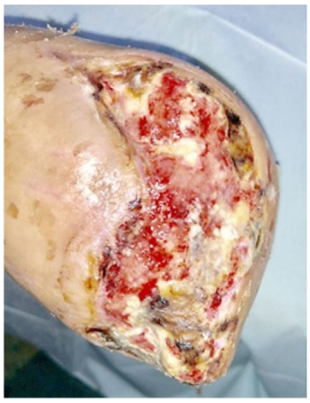

Figure 1 – ***The left foot seven months after the amputation and after the necrotic tissues' removal.***

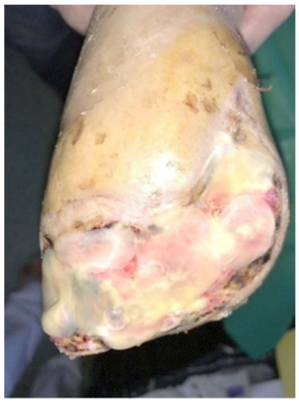

Figure 2 – ***The modality of PRGF application.***

Every two weeks, a routine evaluation of the Hb level was performed, and anemia was corrected through the modulation of erythropoietin (EPO) dosage (Darbepoetin 40 μg/week) and intravenous iron administration (100 mg every other week, before initiation of the PRGF treatment, and 100 mg weekly, during and after the PRGF treatment).

For the histopathological study of the local tissue progression, the fragments of skin biopsy, harvested before and after applying the local treatment with PRGF, were fixed in a 10% neutral buffered formalin solution for 24 hours and paraffin embedded. After sectioning with the microtome, the histological sections were stained using the routine Hematoxylin–Eosin (HE) staining and the trichrome staining based on green-light [the Goldner–Szekely (GS) technique], while the following antibodies were used for the immunohistochemistry studies: anti-cluster of differentiation (CD)3 (monoclonal mouse anti-human CD3, clone F7.2.38, 1/50 dilution, Dako), anti-CD20 (monoclonal mouse anti-human CD20cy, clone L26, 1/50 dilution, Dako), anti-CD68 (monoclonal mouse anti-human CD68, clone KP1, 1/100 dilution, Dako), anti-alpha-smooth muscle actin (α-SMA) (monoclonal mouse anti-human SMA, clone 1A4, 1/100 dilution, Dako), and anti-CD34 (monoclonal mouse anti-human CD34 class II, clone QBEnd-10, 1/50 dilution, Dako).

Results

After necrectomy, the large surface of the wound became significantly larger, requiring a strict monitoring. The paraclinical parameters just before the PRGF use, during its use and after we stop this therapy, are presented below (Table 2).

Table 2 – ***Paraclinical monitoring over the entire period of hospitalization***

Parameter	Before initiation PRGF treatment	During PRGF treatment	After treatment
kT/V (*N*: 1.5)	1.45	1.5	1.5 ↗
Hemoglobin [g/dL]	11.3	11.1	11.5 ↗
PTH [pg/mL] (*N*: 65 pg/mL)	870	840	860
Calcium [mg/dL]	9.4	8.7	8.9 ↘
Phosphate [mg/dL]	4.6	6.5 ↗	6.2 ↘
HbA1c [%]	6.9	6.7	6.2 ↘
CRP [mg/L] (*N*: 0–5 mg/L)	7	4	2.5 ↘
Glycemia [mg/dL] (*N*: <150 mg/dL)	250	145	130 ↘

CRP: C-reactive protein; HbA1c: Glycated hemoglobin; kT/V: Dialysis efficiency; *N*: Normal values; PRGF: Plasma rich in growth factors; PTH: Parathormone.

These data highlighted the fact that almost all investigated parameters had significantly better values after treatment. The imbalances of calcium phosphorus metabolism, with the augmented secretion of PTH, that consequently determined hypercalcemia and possible vascular calcification (due to calcium stored on vessels walls), were controlled by the daily treatment with Sevelamer; this drug removes phosphate from the blood and thus, it may reduce the high values of PTH. This approach is useful in hypercalcemia therapy when calcium chelators cannot be used.

The biopsy performed before the PRGF treatment revealed large areas of recent and more mature granulation tissue. The recent granulation tissue contained many neo capillaries, heavily infiltrated with chronic and acute inflammatory cells (Figure 3a), whereas, the more mature granulation tissue, both fibroblastic and collagenous, contained fewer capillaries (Figure 3b), being well correlated with the long seven-month repair process. At the periphery of the lesion, the epidermis presented areas of hyperplasia (within the regeneration process), with hyperkeratosis, acanthosis and marked elongation of the epidermal ridges. Underneath the epidermis, lymphangiectasia was visible, as well as muscular arterioles in the deep dermis, without luminal obstruction (Figure 3c).

After the first two weeks of the PRGF use, the clinical aspect of the wound improved, with the formation of a peripheral epithelial tissue of approximately 4 mm width and the local development of a granulation tissue (Figure 4).

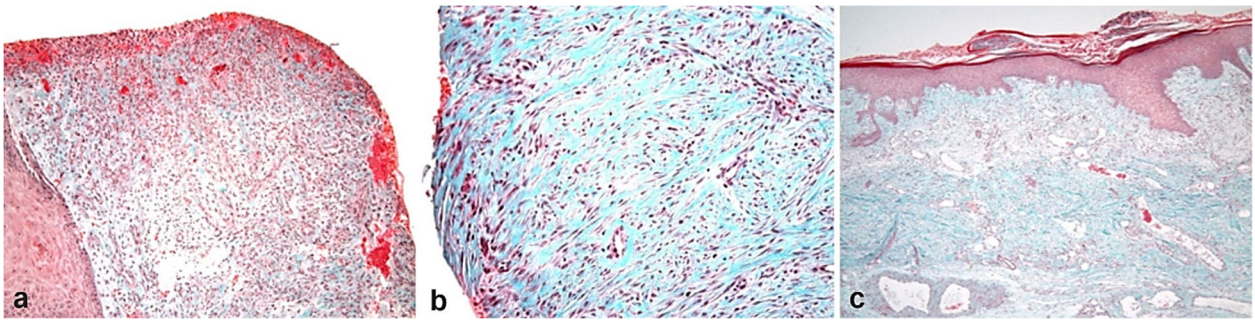

Figure 3 – ***Photomicrographs of the biopsy: (a) Recent granulation tissue, with a large number of neocapillaries, neutrophils, lymphocytes, plasma cells and macrophages, covered by a fibrinopurulent exudate; (b) Mature granulation tissue consisting in few neocapillaries, fibroblasts and collagen fibers; (c) Skin from the periphery of the lesion, with the epidermis presenting hyperkeratosis, acanthosis and elongation of the epidermal ridges, dermis with slight , visible between the superficial dermis and the reticular dermis and free dermal muscular arterioles. Masson's trichrome staining: (a) ×100; (b) ×400; (c) ×40.***

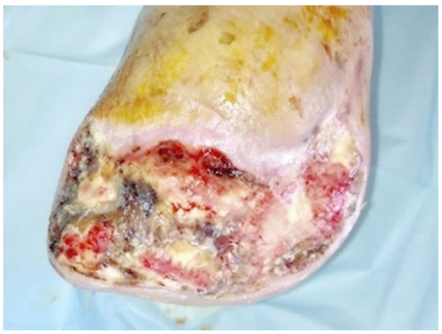

Figure 4 – ***The wound after two weeks. At the periphery of the wound, there is an incipient process of re-epithelization.***

Two weeks after applying the PRGF treatment, the microscopic examination showed an intense proliferation of the surface epidermis, with some areas of cellular hyperplasia and deep epithelial apices in the upper dermis. In the upper dermis, there were also observed the presence of a young granulation tissue, with numerous angiogenesis vessels and the reduction of the inflammatory cell number. In the deep area of the dermis, there were highlighted the presence of numerous fibroblasts and a high collagen synthesis (Figures 5 and 6).

Immunohistochemistry studies highlighted the presence of a moderate inflammatory infiltrate, heterogeneously spread into the granulation tissue, containing numerous T-lymphocytes, macrophages, and fewer B-lymphocytes (Figures 7–9). The anti-α-SMA antibody immunomarking highlighted the presence of numerous myofibroblasts in the granulation tissue, together with numerous blood vessels like arterioles and venules for highlighting the smooth muscular cells from the structure of these vessels (Figure 10). Also, anti-CD34 antibody allowed us to highlight the presence of a very large number of small-sized blood vessels (capillaries, arterioles, and venules), with hypertrophied endothelial cells, presenting an angiogenesis aspect (Figure 11).

Our study showed that the PRGF treatment favors tissular regeneration processes by stimulating the multiplication of fibroblasts and myofibroblasts, cells that produce a conjunctive matrix, especially collagen fibers, increase of blood vessel network by stimulating the angiogenesis process, reduction of inflammatory infiltrate, a more rapid maturation of granulation tissue and a good re-epithelization of wounds (Figure 12).

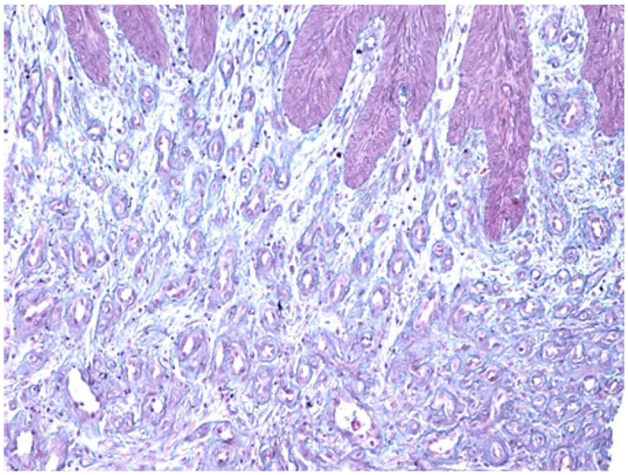

Figure 5 – ***Image of upper dermis, formed of young granulation tissue, with numerous angiogenesis vessels and a low quantity of inflammatory cells. GS trichrome staining, ×100. GS: Goldner–Szekely.***

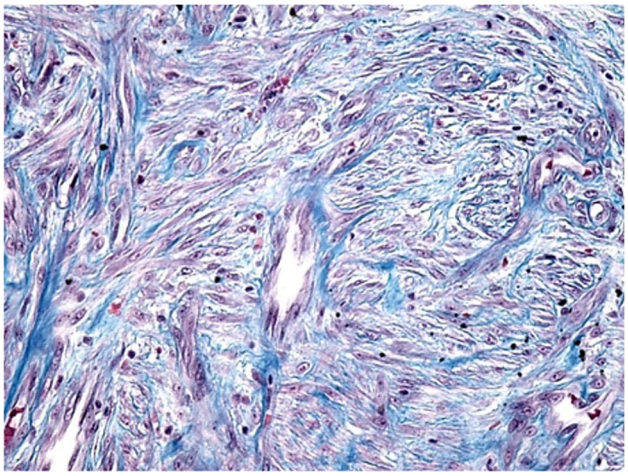

Figure 6 – ***Deep dermis rich in fibroblasts and collagen fibers with a tendency of fascicle formation. GS trichrome staining, ×200.***

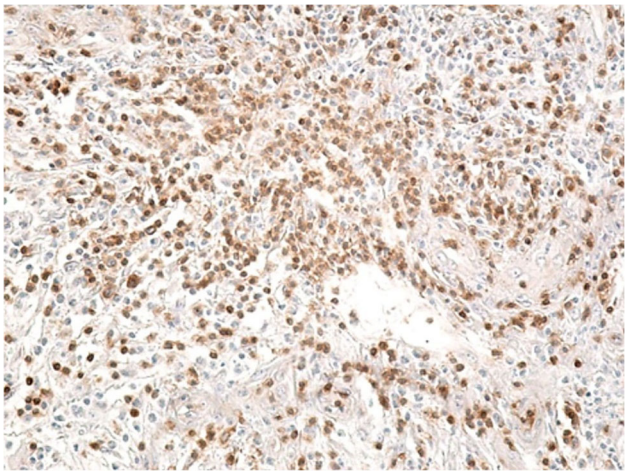

Figure 7 – ***Area of granulation tissue, with a rich inflammatory infiltrate, mainly formed of CD3-positive T-lymphocytes. Anti-CD3 antibody immunomarking, ×100. CD3: Cluster of differentiation 3.***

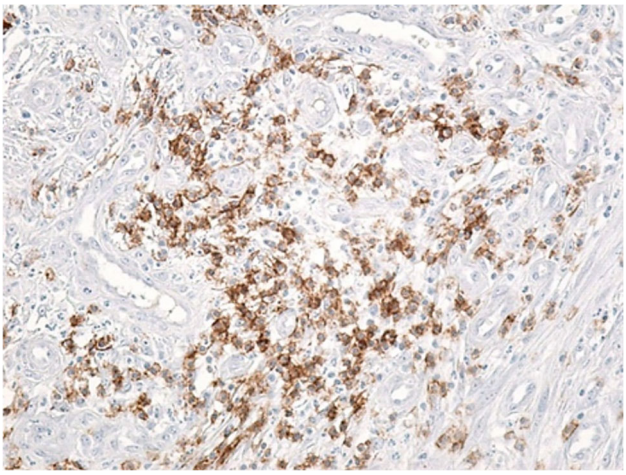

Figure 8 – ***Moderately infiltrated granulation tissue with CD20-positive B-lymphocytes. Anti-CD20 antibody immunomarking, ×200. CD20: Cluster of differentiation 20.***

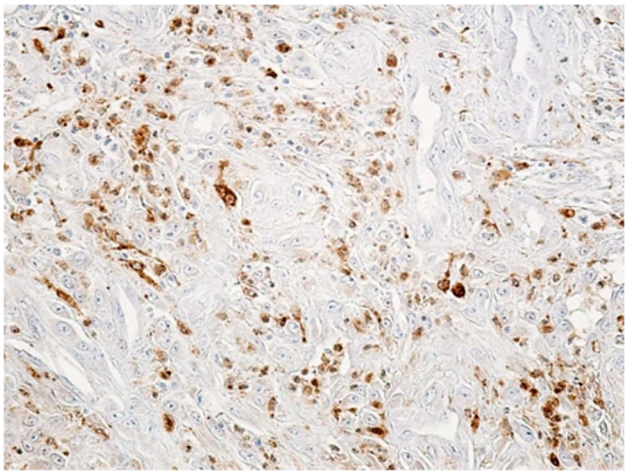

Figure 9 – ***Area of granulation tissue infiltrated with a high quantity of macrophages. Anti-CD68 antibody immunomarking, ×100. CD68: Cluster of differentiation 68.***

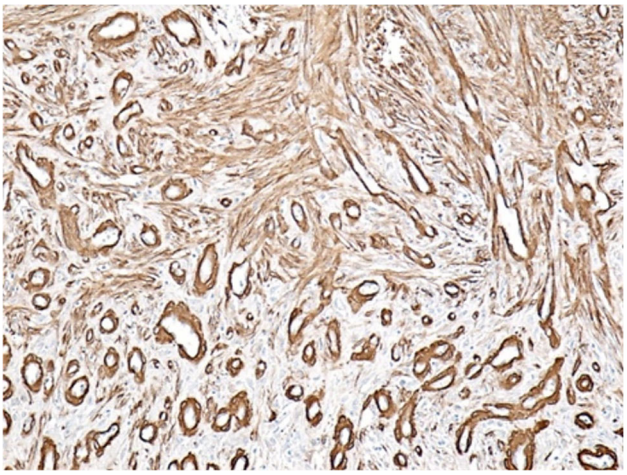

Figure 10 – ***Granulation tissue rich in myofibroblasts and blood vessels. Anti-α-SMA antibody immunomarking, ×100. α-SMA: Alpha-smooth muscle actin.***

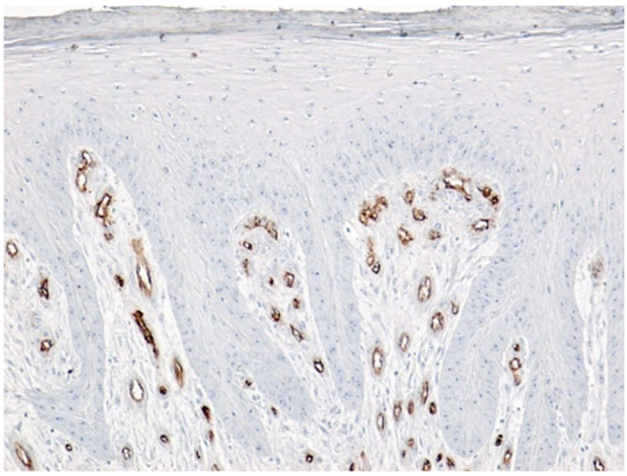

Figure 11 – ***Microscopic image from an area of complete re-epithelization of the wound, where there is observed the presence of a high number of blood vessels (arterioles, capillaries, venules) in the upper dermis. Anti-CD34 antibody immunomarking, ×100. CD34: Cluster of differentiation 34.***

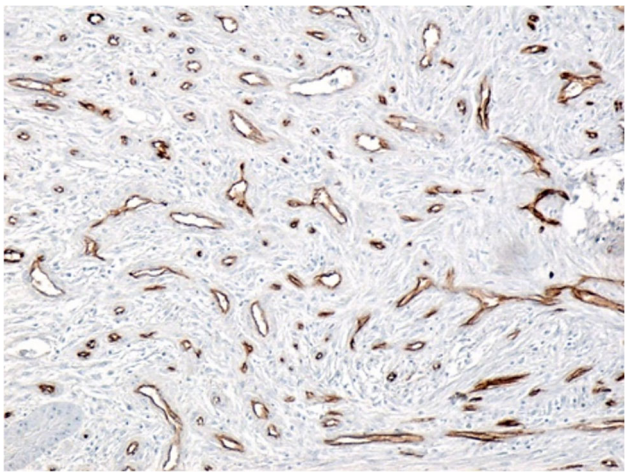

Figure 12 – ***Granulation tissue in the deep dermis, during the maturation process, with a dense network of blood vessels. Anti-CD34 antibody immunomarking, ×100.***

A month later, there was recorded a stagnation in the epithelization of the wound, the culture revealing the presence of *Enterococcus*. After concomitant use of PRGF and Amoxicillin based on the antibiogram for 10 days, the process of healing continued. Two months later, half of the wound was covered by an epithelium and three months later, the wound was almost completely healed (Figures 13–15).

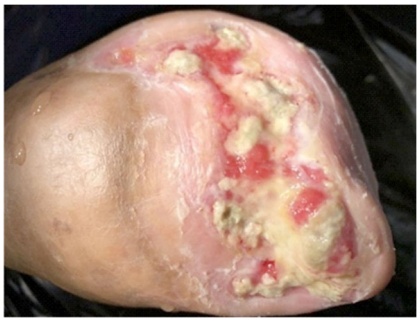

Figure 13 – ***Macroscopic appearance of the lesion after one month of PRGF use. It is observed the reduction of the areas of tissue necrosis, the development of the granulation tissue and the epithelization of the wound on a very large surface. PRGF: Plasma rich in growth factors.***

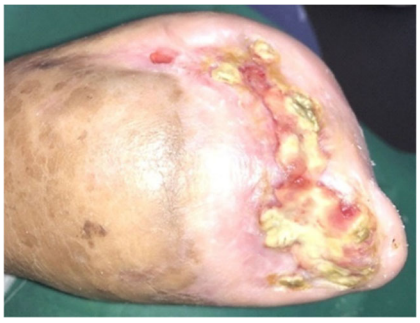

Figure 14 – ***Image of the wound after two months of treatment. There is a favorable evolution, with almost complete recovery of the skin.***

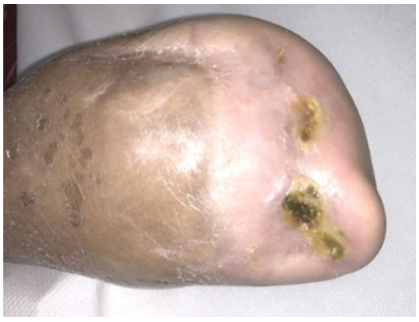

Figure 15 – ***The evolution of the healing process after three months after using PRGF. The complete restoration of the skin that covered the wound can be seen. PRGF: Plasma rich in growth factors.***

During all this time, the surface of the wound was bacteriologically negative. The level of Hb did not decrease significantly during the procedure, with an average value of 11.3 g% under the similar EPO doses. The slightly higher values of blood sugars (less than 150 mg%), did not interfere with the beneficial effects of PRGF.

Considering the reduced surface of the wound, we decided to discontinue the administration of PRGF, with consecutive monitoring. However, five months later, the wound was still not completely closed, and the presence of *S. aureus* was revealed on the surface of the wound. A two weeks' treatment with Vancomycin was initiated, adjusted to the renal dysfunction. Consecutively, the surface of the remained wound slowly decreased until its complete disappearance within a month.

Discussions

The vascular pathology in the patients with renal failure has multiple causes, from arteriosclerosis to atherosclerosis which is associated with the built up of fatty plaques, amyloid and calcium crystals deposits [18]. Consequently, an occlusive arterial disease, with various locations could occur [19]. When a peripheral artery is involved, mainly in diabetic uremic patients, an acute ischemia can occur and skin ulcers are frequently formed; healing is difficult, often requiring amputation, and the patient needs a prolonged hospitalization, with high costs for drug therapy and transport to hemodialysis, resulting in a low quality of life.

The complex process of wound healing can be separated into three or four stages: (*i*) hemostasis, (*ii*) inflammation, (*iii*) cell proliferation, and (*iv*) remodeling, which are monitored by various types of cells that release GFs and cytokines; the latter can directly act on the nearby or even distant cells, responsible for triggering their release [9, 20]. Due to their essential role in the process of tissue healing and regeneration, the GFs are crucial in wound therapy [9, 21].

The GFs as well as their role in wound healing are shown in Table 3 [22].

Table 3 – ***Growth factors involved in wound healing***

Stage of healing	Factors involved
Inflammatory phase	G-CSF, TGF-1, TGF-2
Proliferative phase	PDGF, FGF, VEGF
Epithelization	EGF, KGF, GM-CSF
Remodeling phase	TGF-β3

EGF: Epidermal growth factor; FGF: Fibroblast growth factor; G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor; KGF: Keratinocyte growth factor; PDGF: Platelet-derived growth factor; TGF: Transforming growth factor; VEGF: Vascular endothelial growth factor.

The PRGF–Endoret® used in this case is an autologous product containing a moderate concentration of platelets and free of leukocytes since the inclusion of leukocytes increases inflammation and pain and augments the damage of the fibrin [17]. PRGF promotes the development of a biological scaffold consisting of fibrin enabling cell adhesion, which is favorable for wound healing process, due to the gradual and balanced release of a wide variety of molecules, including GFs and other proteins [17, 23]. The main advantages of this system are the followings: it is a biocompatible, versatile (adapted to the patient's clinical needs) and safe product (no risk of rejection, no adverse effects); the absence of leukocytes prevent their proinflammatory effects; it provides a controlled activation of platelets using calcium chloride for the release of GFs; it is reproducible, based on an easy and fast protocol (involving centrifugation for eight minutes and preparation for 20 minutes) [9, 17, 24].

PRGF has a significant role in tissue regeneration, due to the GFs, which stimulate the proliferation of fibroblasts, the production of collagen I and III, the osteoblast progenitor cells and the synthesis of bone collagen, but also induce the apoptosis of the osteoclasts [23, 25]. Platelets contained in this product have an essential function in angiogenesis by releasing various proangiogenic factors, such as: vascular endothelial GF (VEGF), epidermal GF (EGF),

fibroblast GF (FGF), platelet-derived GF (PDGF), and angiopoietin-1 (Ang-1). In addition to its mitogenic, differentiating and angiogenic effects, PRGF also exerts an antibacterial effect against *S. aureus* and *Escherichia coli* [26].

The patients in hemodialysis program exhibit several particular aspects: (*i*) during one week, due to the succession of hemodialysis sessions, the blood contains a higher or a lower quantity of uremic toxins; (*ii*) they receive an anticoagulant therapy which could interfere with the PRP procedure, including with the formation of fibrin clot; (*iii*) anemia is always present, due to the deficit of EPO and inherent blood loss on the extracorporeal circuit; (*iv*) the inflammatory syndrome is significant; (*v*) high glycemic levels promote favorable growth media for microorganisms infecting the wounds.

Because the hemodialysis procedure modifies favorably the quality of the plasma obtained from the withdrawn blood and standard hemodialysis implies three times/four hours/week, our protocol recommends the application of PRGF to be carried out in between the hemodialysis sessions, and for an ideal quality of plasma, preferable the day after the last hemodialysis of the week. Administration of standard or fractionate heparin is necessary to prevent the coagulation of the extracorporeal circuit at the beginning of hemodialysis. Thus, a quantity of anticoagulant is possible to remain in the bloodstream and to interfere with the application of PRGF. Therefore, PRGF procedure should be applied in the free interval, between the hemodialysis.

The significant anemia is another problem interfering with the use of PRGF. The monthly hematological monitoring will orient the EPO and iron dose administration for anemia correction. In the hemodialysis patients, there is a deficit of EPO, but also a resistance to normalize its level, when applying EPO, due to: the inflammatory syndrome, HPTH, folate deficit, reduced life span of the red blood cells, and blood loss in extracorporeal circuit. Monitoring the hematological parameters (Hb, sideremia and ferritin/transferrin saturation) is carried out as a monthly routine (or twice a month when necessary). Therefore, we recommend that the withdrawing of the blood for PRP to be done once a week, from the peripheral venous circulation.

Patients with ESRD have an increased level of pro-inflammatory biomarkers. Literature data suggest that in ESRD proinflammatory cytokines are essentially involved in the pathogenesis of malnutrition, arteriopathies and other cardiovascular diseases [27, 28]. In clinical practice, CRP is regarded as the most significant marker used in the diagnosis and control of inflammation. CRP is involved in inflammation through its linking to lipoproteins and further activation of the complement system [29, 30]. CRP acts like a mediator for developing atherosclerosis, contributing to atherogenesis by directly affecting endothelial cells, monocytes–macrophages, and smooth muscle cells [31, 32]. The patient in the discussed study had at the very beginning an increased CRP level, but, during the PRGF treatment, the CPR level became normal. In addition, in patients with ESRD, increased levels of other parameters related to inflammation, including erythrocyte sedimentation rate (ESR), tumor necrosis factor-alpha (TNF-α), adiponectin, hepcidin, and ferritin were described; however, serum levels of albumin, low-density lipoprotein (LDL)– and high-density lipoprotein (HDL)–cholesterol decrease within inflammatory process [28, 33]. The levels of other inflammatory markers: adiponectin and TNF-α were higher in hemodialysis patients compared with controls [34]. Hemodialysis gave the opportunity of three times a week examination of the wound. However, we decided to keep the PRGF membranes for four days, applying a flush of sterile normal saline solution at this time, and re-covering it with sterile dressings until the next PRGF applying.

In this patient that suffers from diabetes and hypertension, with a high level of PTH, the underground lesion could have been not only arteriolosclerosis, but calciphylaxis. This disorder is related to obesity, female gender, HPTH, hemodialysis, diabetes mellitus, malignancies, chronic inflammation [35, 36]. In ESRD, all vascular calcifications and all vascular thrombosis are significantly associated with calciphylaxis. Perieccrine calcification is specific to calciphylaxis and, if vascular and extravascular calcification are not visible, this finding could establish the diagnosis [36]. The calcium deposition was best highlighted by Alizarin Red staining [36]. However, the histopathological study in our patient did not reveal the presence of calcium crystals or signs of calciphylaxis.

The use of PRGF, with the increased concentration of GF, and consecutive coverage with fibrin clot proved to be efficient for the healing process of this wound with a deficient vascularization. PRGF application should not be regarded as an alternative to conventional therapy (involving debridement of necrotic tissue), but as an adjunctive therapy [24, 37]. PRGF was used in maxillo-facial surgery to stimulate regeneration of osseous and epithelial tissues [25, 38], in orthopedic surgery, sports medicine [39], arthroscopic surgery [40]. This innovative system also has application in anti-ageing since it stimulates the body's own healing process for rejuvenation. But in human clinical trials, PRGF's main role was to treat chronic conditions, including chronic ulcers. This system was also applied to cure the chronic ulcers in patients with acquired immunodeficiency syndrome (AIDS) [41].

In the literature, there are some prospective studies showing the effect of PRGF on diabetic wounds, but none of these cases presented a hemodialysis diabetic patient treated by this procedure [42, 43]. Considering the complex pathology of the patient as well as the fragmentation of the life-rhythm due to the dependency on hemodialysis three times a week, we had to elaborate a strategy adapted to the particularities of this case. PRGF was applied every week the day after hemodialysis, and the dose was adapted to be efficient for a patient with anemia, in the context of EPO deficiency and inherent blood loss during hemodialysis. The amount of blood initially withdrawn was insufficient to cover the wound with both fractions; therefore, we chose to apply fraction 2 in the medial area and fraction 1 (fibrin) in the lateral area. Later, as the wound decreased in size, fraction 2, which was the most important, covered the entire surface of the wound, and fraction 1 was applied on top, in accordance with the BTI Guide of using PRGF in dermatology [44]. Considering the variable blood glucose levels caused by diabetes, the patient required a thorough control of glycemia before and during the wound treatment (to counteract not only the oxidative stress induced by high blood glucose levels, but also to prevent the wound contamination).

The healing process was early initiated after the application of PRGF and had a favorable progression; however, some delays occurred, caused by wound infection. The newly formed repair tissue was thin, non-pigmented, elastic, and resistant to pressure. After four months, the surface of the wound progressively and constantly decreased. Moreover, one of the evidences supporting the method efficiency is the stagnation in the healing process and the persistence of a small ulcerative lesion, when the PRGF treatment was stopped. The complete healing of the surface of anterior foot took three months, because a lesion of approximately 1 cm^2 persisted with no tendency to heal.

Conclusions

PRGF proved to be efficient in treating difficult after amputation wounds in a diabetic patient, with a complex associated pathology, enrolled in a hemodialysis program, with no negative impact on anemia. Therefore, it is recommended to use this technique in all situations where the healing process of the cutaneous injuries is deficient.

Conflict of interests

The authors declare that they have no conflict of interests.

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