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A multistage combined approach to promote diabetic wound healing in COVID-19 era

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Abstract

When diabetes mellitus is not properly controlled with drugs and a healthy lifestyle, it exposes patients with advanced peripheral arterial disease or critical limb ischaemia (CLI) to the most serious complications, in particular lower limb ulcers. Surgical or endovascular treatments represent the first line of intervention; in addition, the adequate management of ulcers can guarantee not only a faster wound healing but also the improvement of the patient's prognosis. To speed up this process, negative pressure wound therapy (NPWT), platelet-rich plasma (PRP), and other advanced moist wound dressing have been proposed. During Coronavirus disease 2019 (COVID-19) pandemic, many patients with CLI and diabetes mellitus had difficult access to advanced treatments with a significant reduction in life expectancy. We report the cases of patients with non-healing ulcers and CLI treated with an empiric multistage approach after successful endovascular revascularisation; the postoperative course was eventful in all patients, and foot ulcers are currently in an advanced state of healing. The association between adequate revascularisation, systemic anti-inflammatory, and antibiotic therapy with the multistage advanced medications ensures healing of ulcers, limb salvage, and improvement of patient prognosis.

KEYWORDS

CLI, diabetes mellitus, non-healing ulcers, NPWT, PRP

1 | INTRODUCTION

Patients with diabetes and critical limb ischaemia (CLI) are chronic generally, and their history is often characterised by numerous hospitalisations for frequent complications such as infection, neuropathy (chronic severe pain), and foot ulceration.[1] Recently we showed the pivotal role of negative pressure wound therapy (NPWT) and different moisture dressings as advanced adjuvant therapy after adequate revascularisation[2] in patients with CLI[2-5]; moreover, several authors[6] showed that autologous platelet-rich plasma (PRP) applied in patients with diabetes and CLI undergoing minor amputation can be effective to improve wound healing. In other important work, they also showed that PRP associated to skin graft procedures can be effective to speed up the healing rate of non-healing ulcers.[7,8] Despite the numerous evidences, unfortunately, it is not always easy to manage complications due to poor patient compliance and poor adherence to follow-up protocols.[9,10] For these reasons, some wounds may show no progress in the healing process especially in the case of diabetes.

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We report two cases of refractory to treatments non-healing infected diabetes foot ulcers treated with a "multi staged—multi component" approach during Coronavirus disease 2019 (COVID-19) era.

2 | CASE REPORT

2.1 | Patient 1

A 73-year-old Caucasian male patient was admitted to our Institution for a worsening rest pain on the lower left limb. Seven months earlier, the patient underwent coronary artery bypass grafting and he developed, at the distal middle third of limb (near the harvesting area of the great saphenous vein), a severe infection of the anterior tibial lodge, which resulted in a large eschar with exposure of the anterior tibial tendon. Numerous attempts to improve the local state at home were ineffective, and the pain was very difficult to treat.

Medical history was remarkable for uncontrolled systemic hypertension and diabetes mellitus, severe chronic kidney disease with multiple brachial artero-venous fistulas on both superior limbs rapidly thrombosed over the years and final haemodialysis access in the right subclavian vein with Shaldon device, dyslipidemia, previous revascularisation of right superficial femoral artery with percutaneous transluminar angiography (PTA)/stenting, and associated transmetatarsal amputation on the right limb.

A control B-mode ultrasonography and colour imaging showed a diffusely severe femoral artery calcifications involving tibial arteries with severely demodulated flows.

An endovascular approach was chosen. Initial arteriography showed multiple calcific severe stenoses of the superficial femoral artery that are treated with PTA (5 mm medicated balloon); moreover, the severe stenosis of the posterior tibial artery located to the middle distal third, and the occlusion of the anterior tibial artery located to the middle third are also treated with PTA (2 and 2.5 mm balloons). The final angiography showed the patency of superficial femoral artery and the main run-off vessels and a good plantar circulation was restored.

After 6 days from the operation, the large ulcer on the tibial region was treated with deep escharectomy, tendon's section of the anterior tibial muscle and meticulous removal of the necrotic tissues; furthermore, post-operative swabs showed no important bacterial species (Figure 1). Two days after surgical debridement, the PRP gel was applied topically and covered with non-adherent dressings and sterile gauze, as previously described.[11] It was applied twice a week for 3 weeks for a total of six applications.

Key Messages

- In the COVID-19 era, many patients with diabetes mellitus and CLI had difficult access to advanced treatments in hospitals, and therefore, they often developed non-healing diabetic ulcers
- An altered balance between metabolic status, the severity of peripheral arterial disease, and inadequate systemic anti-inflammatory therapy may influence the healing process of minor amputation and escharectomy following endovascular revascularisation
- The use of NPWT, PRP, or other advanced moist wound dressing has been proposed to treat the non-healing wounds, but sometimes diabetic ulcers are refractory to treatments
- To enhance the effect of PRP, the intra-wound injection associated with NPWT and periodic debridement improved the state of non-healing diabetic ulcers and ozone therapy improves the local state of the ulcer with anti-inflammatory and antibacterial effect
- Local aggressive management of the wound with multistage medication (NPWT with intra-wound PRP—ozone Therapy—debridement) after successful endovascular revascularisation associated with systemic anti-inflammatory therapy speed up the wound healing process improving the prognosis of patients with diabetic ulcers

The patient was treated with antiplatelet drugs, statins, and systemic antibiotic therapy, and targeted therapy to decrease glucose levels was set.

Despite all, in the following days, the ulcer became more and more exudative and its size did not decrease. The patient contracted a new infection with swabs positive for *Candida albicans*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Staphylococcus epidermidis*. The patient also became feverish further surgical debridement was needed because of a severe bacterial infection of postoperative ulcer with a high level of Reactive C-protein (RCP) (from 58 700 to 148 200 μg/L).

NPWT was chosen to treat the non-healing infected ulcer in association with the intra-wound PRP injection (Figure 2).

2.2 | PRP liquid preparation

Platelet-rich plasma was prepared by centrifugation of autologous whole blood at 1200*g* for 5 minutes and removal of leukocytes, erythrocytes, and residual substances. Subsequently, PRP was activated with 10% calcium citrate and injected 0.05 mL/point/cm^2 into the prepared wound using 30-gauge needles.

FIGURE 1 After escharectomy and section of the anterior tibial muscle tendon. The wound bed preparation with the removal of necrotic tissues and bruising of soft tissues to promote the growth of granulation tissue was performed

FIGURE 2 Wound bed status after the injection of liquid PRP and NPWT with bagging-like local ozone therapy. The wound appears clean without signs of bacterial infection, and its size is reduced with non-congested edges, the granulation tissue appears luxuriant and the avascular tissues are entirely covered

2.3 | Ozone preparation

Ozone was generated by an ozone generator at concentrations of 5–80 mg/mL at a flow rate of 1 L/min. Ozone therapy was performed at a concentration of 75 mg/mL.

NPWT-PRP liquid was performed three times a week for 4 weeks and an empiric ozone bagging-like therapy was directly applied into the connection pipes of NPWT machine (temporarily blocked), for 15 minutes, every day, 5 days a week (Figure 3). Before any dressing, an accurate debridement was done.

After a few days, the wound bed showed several signs of active and adequate granulation, peri-lesional became less pronounced and the wound swabs became sterile for any bacterial species. RCP levels were drastically reduced (12 000 μg/L).

After 10 days from the first combined treatment (intra-wound PRP/NPWT + ozone), a skin graft was done (Figure 4) and, as previously described,[8] we decided to continue the PRP application for further days, twice a week.

The patient was discharged in good haemodynamic conditions, and the complete wound healing was achieved after 1 month.

2.4 | Patient 2

An 80-year-old male patient was admitted to our Emergency Department for a sub-acute right limb ischaemia

FIGURE 3 Skin grafting associated with PRP injection in the operating room. Before grafting, wound bed preparation consisted of surgical debridement

FIGURE 4 After 10 days of treatment. The skin graft achieved excellent engraftment on the wound bed. There are no signs of necrosis or other tissue sufferings, nor of bacterial superinfection. The limb is not oedematous and the edges of the wound are not altered

arisen for about 10 days; marked cyanosis of the III and IV rays characterised his limb. Medical history was remarkable for previous myocardial infarction treated with Percutaneous Transluminal Coronary Angioplasty (PTCA), uncontrolled diabetes mellitus and systemic hypertension, chronic atrial fibrillation treated with anti-arrhythmic, and oral anticoagulative therapy.

A control B-mode ultrasonography and colour imaging showed the occlusion of the superficial femoral artery and a poor distal flow of popliteal artery supported by genicular collaterals; the anterior and posterior tibial arteries also had very poor flow and the peroneal artery was difficult to sample due to important shadow cone. The patient underwent urgent angiography that confirmed the occlusion of the superficial femoral artery, the tibial vessels were not well visualised and the peroneal artery presented critical stenosis on its origin. Catheter-directed therapy, including endovascular clot aspiration, was needed and the PTA/stenting of the distal femoral artery and the peroneal artery was performed. The final angiography showed regular vascularisation of the deep femoral artery and patency of previous treated femoral and popliteal arteries and tibioperoneal trunk; however, at the level of the middle third of the leg, an occlusion of anterior tibial and peroneal arteries was reported probably due to distal embolisation. The peroneal artery was

FIGURE 5 Diabetic ulcer not responsive to NPWT. The edges of the wound show signs of tissue suffering with bacterial superinfection; the medial surface of the fifth finger shows abundant deposition of fibrin compatible with ischaemic suffering and evolution towards wet gangrene

hardly re-inhabited at the level of the distal third of the leg by the anterior tibial artery; the pedal artery was partially inhabited by collateral vessels. For this reason, the placement of a lytic infusion catheter just beyond the origin of the peroneal artery was needed to ensure the resolution of microemboli in distal portions of the foot's vessels. After 48 hours, angiographic re-evaluation showed an adequate run-off flow of distal vessels.

In the following 10 days, the marked cyanosis evolved in dry gangrene of part of the forefoot, which required an atypical trans-metatarsal amputation with the removal of III and IV rays. Two days after, NPTW was packaged to enhance the granulation process of the wound.

Systemic anti-inflammatory therapy with antiplatelet drugs and statins was set, in addition to the correct dosage of anticoagulant drugs. Due to the initial poor compliance of the patient, glucose levels were poorly controlled for about a week, despite supportive therapy. For this reason, despite the periodic debridements, the wound showed no signs of healing, and after a few days it began to be exudate, with congested edges and a fibrous and purulent bottom; moreover, an unresponsive to medications superficial wet necrosis began to develop on the medial surface of the V finger (Figure 5). The wound swab showed *Candida albicans* and *Klebsiella pneumoniae*, the latter multi-resistant, and a targeted antibiotic therapy was set up. R-CP levels increased from 26 000 to 123 000 μg/L. The patient underwent another surgical redo-debridement in the operating room to treat the important infection and a partial amputation of the V ray with residual flap rotation was needed. NPWT associated with an intra-wound PRP injection was started and it was performed three times a week analysing the

FIGURE 6 Intra-wound injection of PRP associated with NPWT. The size of the ulcer is reduced, the edges no longer show signs of bacterial superinfection, and the bottom of the wound is characterised by granulation tissue in an advanced phase of activity. The little flap obtained from the partial amputation of the fifth finger has taken root well in the wound bed and there are no evident signs of ischaemic suffering or infection

FIGURE 7 After combined therapy. The application of the local ozone associated with the intra-wound injection of PRP allowed to obtain a rapid granulation process with a substantial reduction in the size of the ulcer, disappearance of pain and resumption of patient mobility

microbiological state of the wound and the R-CP values after every injection (Figure 6). Moreover, we started the ozone therapy directly applied into the connection pipes of the NPWT machine temporarily blocked, every day, 5 days a week.

After 2 weeks of combined treatments, RCP values were dramatically reduced (from 80 000 to 16 000 μg/L), the wound swabs were also negative for the multidrug-resistant species and the state of the wound drastically improved with a good granulating bottom, non-hyperemic edges, the almost total disappearance of the fibrin bed; moreover, the rotation flap took root very well at the bottom of the wound (Figure 7).

The patient was discharged after 10 days in good metabolic status and adequate clinical and haemocoagulative control, and the diabetic ulcer showed an advanced state of granulation (Figure 8). The non-healing wound healed definitively after about 1 month.

FIGURE 8 10 days after. The almost complete coverage of the lesion has been obtained; the bottom of the wound is in an advanced state of healing and the process of re-epithelialisation by the second intention is in progress

3 | DISCUSSION

The natural history of patients with diabetes and CLI is characterised frequently by foot ulcers onset during their life (approximately 15%-25% of all ones)[12] and often a great percentage of these are infected and represent the major causal factor for lower limb amputation.[13] The success of open and endovascular revascularisation and the healing of postoperative wounds represent the cardinal points that allow to improve the patient's survival and prognosis.[14-16]

According to the literature,[17] especially in diabetes,[18] another very important aspect is the postoperative wound management that must necessarily be adequate and "aggressive" to ensure healing.[19]

The points above described have been partially disregarded because to the epochal event of which the whole world was a victim: the COVID-19 pandemic. During its critical phase, many hospitals in Italy have intentionally reduced non-emergent elective procedures and hospitalisations to preserve hospital capacity and to ensure treatment of several respiratory symptoms.[20] For this reason, most of the resources, investments, and materials previously aimed for patients with chronic vascular diseases have decreased dramatically[21]; conversely, particular attention was given for ozone medical preparations for its potential role in cytoprotection of organ damage induced by COVID-19.

In our study, we reported two cases of patients with diabetes, poor glucose blood level control, and non-healing ulcers who were hospitalised, with great difficulty, during the critical phase of COVID-19 in Italy with very advanced CLI. An infrapopliteal vessel stenosis or occlusion required in both patients, endovascular revascularisation with PTA of the superficial femoral artery and tibial vessels, as proposed by recent guidelines.[22] No surgical second look procedures of revascularisation are performed thanks to early and mid-term patency of runoff vessels. Moreover, we treated both patients with surgical debridement, escharectomy, and tendon's section and minor amputation, respectively, and we initially obtained an excellent wound bed preparation. Patient 1 was treated with PRP in gel form to take advantage of large amounts of growth factors, which stimulate the production of collagen, modulating inflammation, and extracellular matrix through minimum quantities of plasma.[11] Probably due to a longtime poor metabolic control, the initial inadequate systemic anti-inflammatory therapy (statin and antiplatelet therapy), and lack of dedicated medical staff, after numerous applications, there was no optimal results and ulcers got infected. Similar unsatisfactory results were obtained by the application of the NPWT alone, especially in patient 2, forcing us to identify a new adjunctive modality to promote wound healing and to avoid the need for limb amputation. We decided to experience the intra-wound injection of PRP in addition to NPWT and the local ozone therapy, taking advantage of the large amount of this medication used in our Institute as an empirical line to counteract the most serious respiratory stages of COVID infection.

It is well known that CLI and diabetes are associated with an important systemic inflammatory condition and the dis-regulation of activities of important proteases such as metalloproteinases represent the main point causing the evolution of the disease and the onset of ulcers[23,24]; chronic inflammation and metalloproteinases are the key elements for the development of the most fearful complication of diabetes, namely infection.[25,26] Some evidences seem to rule out a relationship between presence/absence of peripheral obstructive arterial disease and response to PRP gel application in patients with diabetes[27]; specific individual characteristics such as habits and/or the type of blood donor from which PRP is extracted could influence the final results. In our opinion, the clinical effect of PRP gel alone correlated not only with the habits of the patient (hygiene, rest walking, diet compliance, and metabolic control), but also and above all with the "systemic inflammatory power" of the underlying arterial disease.[4] The greater and more adequate is the metabolic and down-regulation of systemic inflammation, the better and faster the response in terms of wound healing.

Few reports on the combination of PRP and NPWT are present in literature probably because in the classic method the plasma is sprayed directly onto the wound and could be drained away by negative pressure.[28] The NPWT-PRP therapy we chose is characterised by intra-wound injection of PRP together with periodical debridement and antibiotic therapy. After just 2 weeks of treatment, especially in patient 1, we documented an unexpected resolution of the peri-ulcerative hyperemic picture, an evident reduction in exudation, and a significant reduction in RCP and erythrocyte sedimentation rate levels. Compared to the PRP gel formulation, the intra-wound injection of PRP can facilitate a better synergy between NPWT and PRP itself, strengthening their common antimicrobial properties, resolving necrotic tissue, enhancing the granulation tissue, and promoting wound healing. An "aggressive" wound management with periodic debridement allows the injected platelet concentrate and related growth factors to take root directly in the less exposed to bacterial attack sites of the soft tissues; in this way, the migratory obstacle for growth factor represented by the infected biofilm[29,30] can be crossed.

Some works showed a possible limitation of antibacterial activity of PRP caused by its short documented action and weakness if compared with antibiotic drugs[30]; in our opinion, an adequate association between different advanced medications is the gold standard protocol to enhance wound healing.[31] In our cases, the periodic debridement allowed effective remediation of the tissues, PRP allowed to keep the wound bed favourable for granulation and NPWT facilitated the safe removal of infected drainage dressing the prior infected ulcers for a resolutive skin graft. Taking advantage of the beneficial effects of NPWT, we decided finally to test the ozone effects on the wound bed status using medical materials available to COVID-19 patients with an alternative bagging approach: the intra-NPWT application through connecting pipes of the sucking system temporarily interrupted. According to evidences,[32] this empirical approach allowed the ozone to act for the 15 to 20 minutes directly in contact with the injured area without the risk of

dispersion or reduction in its effectiveness and without the need to repackage the NPWT dressing. The microbiological swabs performed on ulcers after the "intra-NPWT" administration of ozone showed not only the complete eradication of all bacterial and fungal species but also a clear reduction, up to normalisation, of R-CP levels. These laboratory results had a clinical correlation with rapid reduction of the size of the ulcers, goodness of the granulation tissue, immediate and optimal engraftment of the skin graft, and disappearance of the pain.

The antioxidant and antibiotic effects of ozone have been well studied in vitro experiments[33,34]; despite the excellent results, only a few studies have still verified the efficacy of its local administration in humans.[35] The uncontrolled glucose level in diabetic patients generates high levels of free radicals and antioxidant reduction characterise the mechanisms that may lead to foot ulcer; the homeostasis of the free radical and antioxidant balance control mediated by the ozone therapy was mediated by the modulation of NF-κB/Nrf2 balance and IL-6 and IL-1β expression.[36]

A further element to consider is the type of revascularisation, in fact, our patients underwent endovascular procedures with tibial vessels PTA. Recently we showed that elevated plasma levels of some several circulating biomarkers are strongly related to healing wound and graft patency and the endovascular procedures determine a higher plasma level of the inflammation markers during the follow up probably due to the fact that PTA with or without selective stent positioning may cause endothelial damage or dysfunction greater than a vein graft.[37] Consequently, the systemic and local inflammatory status of diabetic patients with ulcers is decisive in the gradual worsening of clinical conditions, in the difficulty of healing wounds, and in the consequent risk of limb loss.

We can hypothesise that the local anti-inflammatory effect of ozone acted by positively modulating the systemic inflammatory condition induced by the same endovascular procedure; some studies showed a potential role of systemic ozone therapy in patients with foot ulcers compared with other approaches in term of wound healing[35] but the biases were numerous and it is not always clear, which type of revascularisation treatment (open or endovascular?) is chosen.[38]

Further studies on larger patient cohorts are needed to understand the role of systemic ozone therapy in diabetic patients treated with an endovascular approach.

In conclusion, we are confident that the improvement of the metabolic state, the systemic anti-inflammatory treatment therapy and the local synergistic effect operated by the alternation of several advanced dressings with surgical debridement, allowed the patients to get to the wound healing, improving the prognosis decisively.

CONFLICT OF INTEREST

All authors have no conflict of interest.

DATA AVAILABILITY

The data that support the findings of this study are openly available in [repository name e.g "figshare"] at [doi], reference number [reference number].

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