THE “BIOLOGICAL CHAMBER” METHOD – USE OF AUTOLOGOUS PLATELET-RICH PLASMA (PRP) IN THE TREATMENT OF POORLY HEALING LOWER-LEG ULCERS OF VENOUS ORIGIN

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Wound healing is a complex pathophysiological process, in which platelets play a crucial role. Platelet alpha-granules release growth factors to the wound bed; the factors are necessary in the healing process. In chronic wounds, such as poorly healing lower-leg ulcers of venous origin, there is decreased activity of multiple growth factors, so the concept of exogenous delivery of such factors seems a logical strategy. Platelet-rich plasma therapy in patients with lower-leg ulcers of venous origin combined with conventional treatment methods (previously ineffective in these patients) seems, based on our observation, an important adjunct leading to recovery.

The aim of the study was to present an original method of autologous platelet-rich plasma application through the creation of a sort of “biological chamber” containing a concentrate of growth factors. Material and methods. The described therapy was implemented in 10 patients, who had been ineffectively treated for more than one year in the outpatient setting. Patients with exacerbation of inflammatory process, signs of wound infection and ankle brachial pressure index < 0.8 were excluded from the study. After the application of platelet-rich plasma, further treatment was continued with the use of moist therapy and compression therapy according to a uniform regimen.

Results. Complete healing was achieved within 4–10 weeks from the beginning of the product administration in all patients.

Conclusion. The presented method seems technically simple, effective and relatively inexpensive.

Key words: chronic lower-leg venous ulcer, autologous platelet-rich plasma

Lower-leg venous ulcers are a serious complication of chronic venous insufficiency, untreated varices of the lower-extremities and deep vein thrombosis. The repair process in lower-leg venous ulcers is disturbed, abnormal, usually in a chronic inflammatory phase, and one of significant elements of such abnormalities is deficiency of growth factors in the wound bed.

If the undertaken treatment is to be effective, it must be preceded by full diagnostics of the venous system, contain elements of the
treatment of the underlying condition and involve dynamic topical management. One of the factors possibly contributing to faster and more effective healing seems to be topical application of growth factors (1, 2, 3).

Autologous platelet-rich plasma (PRP) is the source of many growth factors playing a key role in the process of normal wound healing, such as: platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) (3, 4). Growth factor supplementation through topical application of autologous platelet-rich plasma in the form of a gel may normalise inflammatory process, with beneficial impact on cell proliferation, and in consequence lead to complete healing of the wound. Therefore, this method may present the patient with another opportunity of improved healing, which in some cases makes it possible to avoid amputation of the limb.

The aim of the study was to present the original method of treating lower-leg ulcers with the use of autologous platelet-rich plasma through the creation of a sort of “biological chamber” containing a concentrate of growth factors obtained from the patients’ own blood.

MATERIAL AND METHODS

The study included 10 patients (7 women and 3 men) with the mean age of 68.3 years treated for poorly healing lower-leg ulcers caused by chronic venous insufficiency (Ethics Committee’s agreement No KNW/0022/KB1/133/I/08). The treatment was applied to chronic wounds – previously treated for more than 6 months, with the mean ulcer area of 10.7 cm². The area of ulcers was measured using our own method of digital documentation of ulcers, which comprises our own wound photography technique, digital processing of the acquired images and our own method of area measurement in digitally processed images using the “Pole” software (5). Patients with exacerbation of inflammatory process, signs of wound infection and ankle brachial pressure index (ABPI) < 0.8 were excluded from the study. The patients were previously operated (they had undergone at least saphenectomy) and treated for more than one year in the outpatient setting without improvement. All patients, before receiving hospital treatment, underwent lower-extremity ultrasound examination and had ultrasonographically confirmed venous insufficiency, which was the direct cause of the underlying condition.

After admission to hospital, samples for bacteriological tests were collected from chronic lower-leg venous ulcers from all patients. In addition, they all underwent mechanical debridement of the wounds (fig. 1).

After topical cleansing of the lower-leg venous ulcer area from necrotic tissues and appearance of granulation tissue at the bottom of the wound as well as after obtaining of negative bacteriological cultures, the wounds were covered with stoma paste in the operating room setting (fig. 2 and 3).

Non-inflammatory foil was placed on the wound and previously applied paste, leaving a wider margin of normal skin of approx. 2 cm (fig. 4).

To the resulting “chamber” autologous platelet-rich plasma was introduced in the form of injection applied to the bottom of the ulcer (fig. 5 and 6). In order to obtain growth factor concentrate, blood was collected from the patients’ basilic vein into 2 syringes containing an anticoagulant – sodium citrate. The blood was then injected into 2 special containers and centrifuged for 15 minutes at 3,200 rpm. Next, platelet-poor plasma (PPP) was removed from the containers using a syringe. The containers were shaken for 30 seconds using circular movements, and then the plasma rich in platelet and leukocytes was collected from each container using one syringe. Thrombin was added to the concentrate. A
The “biological chamber” method in the treatment of lower-leg ulcers

A centrifuge was used as well as equipment produced by Biomet, US in the form of a disposable platelet isolation kit GPS II. Each patient underwent a one-time collection of 108 cm³ of venous blood, which produced 10-12 ml of autologous platelet-rich plasma.

Application of autologous platelet-rich plasma, which was of gel consistency, to extensive lower-leg venous ulcers was not associated with technical difficulties. The gel filled the bottom of the wound tightly, and the foil kept it in constant position.

The lower leg was wrapped in a layer of cotton, after which compression with elastic bandage was used in line with the principles of graduated compression. The method used made it possible to maintain complete isolation from the environment of the dressing with platelet-rich plasma for 3–5 days. After this period the dressing was changed, and the gel reapplied in the way described above. The method of administration of platelet-rich plasma and application of dressings, did not, in patients’ opinion, limit their everyday activity and was well tolerated. Then the ulcers were treated until full healing in line with the principles of the moist therapy and compression therapy.

Fig. 2, 3. Application of stoma paste to the edges of an ulcer with granulation tissue

Fig. 4. Covering of the wound and previously applied paste with a non-inflammatory foil with leaving a margin

Fig. 5, 6. Application of platelet-rich plasma to the bottom of the ulcer using a syringe
RESULTS

After 4 weeks of treatment, the area of ulcers decreased by approx 56%, and after 8 weeks – by approx. 93%; 8 out of 10 patients recovered. All the treated patients achieved marked regression of the lesions, followed by complete healing of the wounds within 10 weeks since the beginning of the treatment.

Subsequent stages of the formation of resorptive granulation tissue are presented in fig. 7-10. The treatment of all patients was complemented from the start with compression therapy, and further steps of healing were conducted according to a uniform regimen. After closure of the wound, further compression therapy was recommended.

DISCUSSION

The treatment consists in topical application of platelet-rich plasma in the form of a gel produced by centrifugation of full blood and addition of a coagulation factor – thrombin.

This concentrated volume of platelets suspended in a small amount of plasma contains many natural growth factors, which are released from a sort of storage – alpha-granules, thus stimulating the healing processes. Chronic wounds are characterised by decreased activity of many growth factors, which has a direct impact on delaying regenerative processes in the wound. That is why exogenous delivery of such factors to the bed of chronic wounds seemed justified. The concept of autologous platelet-rich plasma consists in using the patient’s own blood as an agent improving the healing function of chronic wounds through stimulation of their regenerative potential.

Ross et al. (2) were first to indicate, in their in vitro studies in 1974, that thrombin-activated platelets may act as the source growth factors. This concentrate of platelets in plasma may be directly applied to the wound in order to create there conditions facilitating faster healing.

Autologous platelet-rich plasma stores growth and coagulation factors; it also contains leukocytes and has antibacterial properties.

Fig. 7, 8, 9, 10. Subsequent stages of granulation and wound healing
Addition of platelet-poor serum increased fibroblast activity.

In 1982 Knighton et al. (3) demonstrated, in experimental in vivo animal studies, that thrombin-activated platelet-rich plasma stimulated neoangiogenesis, collagen synthesis, proliferation of epithelial cells and fibroblasts, while the products of fibrin degradation stimulated leukocyte activity. The high concentration of platelets in the plasma guarantees delivery of higher than physiological concentrations of growth factors to the damaged site, which can improve the conditions of tissue regeneration (4, 6). In addition, the leukocytes present in the preparation increase its antibacterial properties (7, 8).

Platelets release to the wound more than 30 growth factors responsible for healing processes, e.g. all three isomers of PDGF (αα, ββ, αβ), VEGF, TGF-β1, epidermal growth factor (EGF), Insulin-like Growth Factor (IGF-1), angiopoietin-2 (Ang-2), interleukin-1β (IL-1β) and more. Autologous platelet-rich plasma also contains proteins responsible for cell adhesion, fibrin, fibronectin, vitronectin as well as osteocalcin and osteonectin (9, 10, 11). Antibacterial properties result mostly from PDGF, which activates macrophages, and VEGF, which stimulates macrophages and monocytes. Fibroblast proliferation is modulated by TGF, stimulation of other growth factors and cytokines is determined mostly by PDGF, while angiogenic activity is exhibited by VEGF and PDGF (12, 13). Growth factors contained in platelets are released through degranulation of the alpha-granules as soon as 10 minutes after the initiation of the blood-clotting processes. Most of them are released within the first hour and bind to the membrane receptors of a nearby cell, activating intracellular signaling pathways (15, 16).

Growth factors may contribute to the process of healing of chronic wounds also through “attracting” undifferentiated cells to the site of the wound and stimulating cell division. Autologous platelet-rich plasma may inhibit cytokine secretion, thus limiting inflammatory reaction, while interactions with macrophages improve tissue healing and regeneration, stimulate growth capillaries and accelerate epithelial repair (17, 18).

After a quick release of growth factors, platelets contained in the platelet-rich plasma synthesise and secrete additional amounts of the factors for 7 days. After this period the healing functions are overtaken by macrophages.

Experimental studies have revealed the most prominent effect of accelerated healing in the 3rd week after the application of autologous platelet-rich plasma (19, 20).

In the extensive literature demonstrating the use of autologous platelet-rich plasma in the treatment of chronic lower-extremity ulcers there are few papers concerning its use in the treatment of lower-leg venous ulcers, although the cause of approx. 80% of lower-extremity chronic wounds is venous insufficiency. It needs to be stressed, however, that none of them questions the effectiveness of autologous platelet-rich plasma in the treatment of lower-leg venous ulcers (21). This method, in combination with the routine management protocol and other additional methods, such as hyperbaric oxygen therapy (HBOT) or maggot therapy (MT), also significantly improves treatment outcomes of diabetic foot ulceration (DFU) (22-27).

Only few papers contain detailed description of the method for application of autologous platelet-rich plasma both in the treatment of lower-leg venous ulcers and other types of ulcers in this location, hence the aim of our study.

An interesting method for application of autologous platelet-rich plasma in lower-leg venous ulcers was presented by O’Connell et al. (28). After standard preparation of platelet-rich plasma from the patient’s own blood, PRFM (platelet reach fibrin matrix) membrane was produced. The process involved a Wheaton bottle, in which platelet-rich plasma was centrifuged for 25 minutes at a high speed in a standard centrifuge. This way the resulting oval PRFM matrix had the thickness of 3 mm and a diameter depending on the volume of collected blood (from 36 ml of full blood a diameter of 50 mm was obtained). The PRFM was prepared in sterile conditions immediately before its application to a surgically debrided wound. The dressing was left in place for 7 days. During 16 weeks of the treatment full healing was experienced by 67% patients with lower-leg venous ulcers. During 16-weeks of follow-up no new complications or adverse reactions were observed. The treatment was complemented with compression therapy.

Crovetti et al. (29) presented a method for application of autologous platelet-rich plasma
in the form of a platelet gel (PG). PG is produced through a complex procedure by mixing activated autologous platelet-rich plasma, cryoprecipitate (frozen plasma) and thrombin. In the case of extensive ulcers in “difficult” locations, a set amount of hyaluronic acid was added to the PG, resulting in easily malleable paste. In 9 out of 24 patients healing of lower-leg venous ulcers occurred after 10 applications, and in 7 patients ulcers were decreased in size by 50%. Dressings were applied every 72 hours.

Cervelli et al. (30) demonstrated an approach different from those presented above. From 18 ml of venous blood, autologous platelet-rich plasma was obtained, calcium chloride was added as activator, and the plasma was immediately mixed with centrifuged subcutaneous adipose tissue collected with special cannulae from the abdominal area. The resulting material was injected in sterile conditions through small incisions around the edges of the lower-leg venous ulcer. After deposition of the material in tissue tunnels, the incisions were closed with 5-0 nylon sutures and no compression therapy was used. In the presented study, out of 20 chronic lower-leg venous ulcers, 16 were healed after 10 weeks. In light of available publications, the use of autologous platelet-rich plasma has significantly increased and improved therapeutic options for healing of ulcers of venous origin.

While the protocol describing preparation of the patients and the wound itself for autologous platelet-rich plasma application was identical in most centres, almost all teams used their own, unique and, as we believe, complex method of application of platelet-rich plasma to ulcers. The essence of the method remained the same; differences pertained to details.

The method for application of autologous platelet-rich plasma should be easy and reproducible. A liquid form of platelet-rich plasma facilitates tissue penetration, but placing it inside a wound is inconvenient. Dense and malleable material decreases tissue penetration, but allows easier placement in the wound. The presented method of application of autologous platelet-rich plasma into a “biological chamber” encompassing the wound bed does not seem technically difficult. At the same time we emphasise the importance of initial mechanical debridement of the wound bed. Owing to the creation of a tight chamber, the liquid form of autologous platelet-rich plasma, easily penetrating into the wound bed, is at the same time maintained at the site of the lower-leg venous ulcer. Platelet-rich plasma used for the treatment was an autologous preparation, so it was not associated with a risk of infection, e.g. with viral hepatitis or HIV. The gel consistency of autologous platelet-rich plasma and its restriction by paste and foil, made it possible to cover the ulcer tightly and create a matrix of sorts, which initiated and enhanced the cascade of repair reactions in the wound bed. Complete healing was achieved within 4–10 weeks from the beginning of the product administration in all patients; further treatment was performed using compression therapy.

The results of our study confirm that the use of growth factors and cytokines contained in platelets as well as leukocytes obtained from the patient’s own blood are an effective and relatively inexpensive method allowing acceleration of chronic wound healing.

Autologous platelet-rich plasma, combined with conventional (previously ineffective) treatment methods, accelerates the healing process of chronic lower-leg venous ulcers, leading to recovery.

CONCLUSIONS

The administration of autologous platelet-rich plasma to a “biological chamber” sealed with paste and foil enables stable application of the preparation to large areas of ulcers, providing good isolation from the environment. It seems that platelet-rich plasma applied in this way is another option of topical treatment in refractory chronic lower-leg venous ulcers, and the presented procedure will facilitate its use.

REFERENCES

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