



EFFECT OF PLATELET RICH FIBRIN ON WOUND HEALING TIME AFTER REVASCULARIZATION

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SUMMARY – One of the most common diseases in vascular surgery is peripheral arterial disease, the last stage of which is critical limb ischemia. It is characterized by rest pain or tissue loss. In most cases, after revascularization, and due to gangrenous changes, it is necessary to perform amputation of a part of the lower limb. After amputation, the wound is bandaged with dressings. In conducting this pilot study, some patients were bandaged with platelet rich fibrin (PRF) in combination with dressings, while the others were treated only with dressings. PRF is a biologic material that is received from blood of the patient. All patients included in the trial had at least 2 out of 3 traversable blood vessels in the lower leg before treatment and underwent femoropopliteal bridging with prosthesis. Patients treated with PRF after revascularization had an average of 5 weeks of wound healing, in contrast to patients treated only with dressings, whose mean wound healing was 8.5 weeks and a proportion of these patients ended up with an extended amputation level. After the study, it is concluded that this type of treatment can be safely used in the treatment of vascular pathology.

Key words: *Peripheral arterial disease; Critical limb ischemia; Platelet rich fibrin; Amputation*

Introduction

By international consensus, critical limb ischemia is defined as the pathological condition of any patient with ischemic pain at rest lasting for more than 2 weeks, ulcers or gangrenous changes with proven arterial steno-occlusive disease¹. Critical limb ischemia itself is the final stage of peripheral arterial disease (PAD), where patients can be classified into stage III-IV (classification according to Fontaine) or into stage IV-VI (classification according to Rutherford²) (Table 1). Risk factors for the development of PAD, i.e., critical limb ischemia, are smoking, hyperlipidemia,

homocysteinemia, hypertension, and obesity². PAD is a rare disease in people under 40 years of age, but the prevalence increases from the sixth decade of life and it affects 25% or more of people aged 80 years. Also, the incidence of this disease can be different in different parts of the world, but in general, it affects men

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Table 1. Fontaine and Rutherford classifications²

Fontaine		Rutherford		
Stage	Clinical	Grade	Category	Clinical
I	Asymptomatic	0	0	Asymptomatic
IIa	Mild claudication	I	1	Mild claudication
IIb	Moderate-severe claudication	I	2	Moderate claudication
		I	3	Severe claudication
III	Ischemic rest pain	II	4	Ischemic rest pain
IV	Ulceration or gangrene	III	5	Minor tissue loss
		IV	6	Ulceration or gangrene

more often than women. Some studies show a higher incidence of PAD in African Americans compared to non-Hispanic and Hispanic white populations². A characteristic clinical picture was observed early, described by Leriche in 1923. The triad of symptoms he listed was called Leriche syndrome, and included claudication in one or both legs, reduced sexual potency (in men), and weakened or absent femoral pulses. Claudications are described as effort-induced cramping pains in the large muscle groups of the lower limb, which are reduced during rest. The progress of the disease gradually shortens the walking distance, and the important moment of the progress of the disease is when the ailments prevent the patient from his daily life activities. The key moment of PAD is pain at rest, which indicates that the patient has developed critical limb ischemia. In the beginning, the pain is intermittent, settles down at night, and then becomes all-day. The patient initially responds to analgesics, and later they lose their effectiveness. Due to inconsistencies in the blood supply and the needs of the extremities, trophic changes occur which can initially manifest as minor wounds (ulcerations), and later as gangrene of the fingers and/or feet with a tendency to spread proximally³. If critical limb ischemia occurs, there is a clear indication for treatment. In patients with PAD, noninvasive vascular measurements and tests can be used. For example, measurement of segmental systolic pressure, segmental plethysmography and measurement of pulse strength (pulse volume recording, PVR), toe-brachial and ankle-brachial index (TBI and ABI), doppler ultrasound, and so-called imaging tests (digital subtraction angiography (DSA), magnetic

angiography (MRA) and computed tomographic angiography (CTA)). Most often, after family history taking and clinical examination, with a detailed vascular status, doppler arterial circulation is used with the measurement of the ABI. The same diagnostic procedure can be repeated at certain time intervals and thus monitor the local vascular status of the patient. Based on the findings of the mentioned tests and the clinical picture, an indication is set for further diagnostic processing (DSA, CTA, MRA), which is indicated if some form of revascularization is planned in the patient⁴. Considering history taking, clinical picture and examinations performed, an indication for conservative therapy or surgical treatment (revascularization treatment) is established. In patients with claudications, if the walking distance is satisfactory, also depending on the patient's age, the treatment is conservative (taking medications such as acetylsalicylic acid, cilostazol and pentoxifylline). Such patients are also advised to do walking exercises to develop collateral circulation. Medical therapy itself, such as cilostazol, has the role of reducing triglycerides and increasing high-density lipoprotein (HDL)². In patients with critical ischemia, revascularization is necessary, if possible. We have at our disposal an open angiosurgical approach, endovascular treatment, or a combination of both, which we call a hybrid procedure. In patients with critical limb ischemia, steno-occlusive changes are often associated infrainguinally with those of the aortoiliac area. In this case, a hybrid procedure is used⁵. In patients with critical limb ischemia, if gangrene of the foot occurs, amputation of the foot is indicated along with revascularization procedure. After the amputation, depending

on the findings of the CTA or DSA, a femoropopliteal (FP) bypass is performed above the knee level with a prosthesis, or FP bypass below the knee level using the greater saphenous vein if it is suitable for creating a bypass. Also, the treatment of such patients is done endovascularly (PTA with or without a stent), or combined⁵. After revascularization, the wound at the amputation site requires daily dressing, and the patient's vascular status must be monitored at vascular department. The speed of wound healing after revascularization is an important event in the treatment process because wound infection often occurs, i.e., spread of the infection process, and thus the level of amputation⁶. Post-operative wound infection is associated with a worse prognosis after revascularization. The World Vascular Society recommends antibiotic therapy for patients with wound infection in general⁷. The TransAtlantic Inter-Society Consensus (TASC) classification can be of great use in the revascularization treatment of patients. TASC is the most authoritative umbrella organization of all the world's largest national multidisciplinary societies, which standardizes procedures on arteries⁴. The TASC group advocates endovascular treatment for TASC type A, and an open surgical approach for TASC type D. For TASC B and C, the authors believe that there is insufficient evidence as to which treatment method is better². Yet, it is important to emphasize that with the progress of endovascular treatment, the TASC classification is increasingly being abandoned, and nowadays TASC type C and D lesions are treated endovascularly. Platelet rich fibrin (PRF) is an autologous material obtained from the patient's platelets and is used to improve wound healing and tissue regeneration⁸. A centrifugation cycle is required to generate PRF, which depends on time, revolutions *per* minute, tube angle relative to rotor axis, and g-force generated by the centrifuge (relative centrifugal force)⁹. The PRF itself is a biologic material obtained from the patient's blood, consisting of platelets, cell fragments and fibrin. The method of treatment with PRF has an important role in plastic surgery, orthopedics, dermatology, pain treatment, sports medicine, cardiac surgery, and urology⁸⁻¹⁰, and this study tried to demonstrate its importance in vascular surgery. Platelet concentrates have also been used in dentistry. PRF has attracted considerable interest from dental community due to its proposed regenerative properties and ability to aid in

wound healing. PRF is proposed to have a direct effect on promoting patient wound healing by supersaturating the wound with growth factors that promote tissue healing. Clinically speaking, PRF is easily produced from the patient's own blood. The autologous nature of PRF makes it preferred over various allografts used in dentistry today. Therefore, PRF has a considerable potential in its applicability in all areas of dentistry, including oral and maxillofacial surgery⁸. In dentistry, test results and meta-analyses confirm that PRF improves the stability of the implant itself. It has also been proven that PRF plays an important role in bone healing and tends to accelerate bone healing¹¹.

We distinguish two types of procedures for obtaining PRF, i.e., advanced platelet rich fibrin (A-PRF) and injectable platelet rich fibrin (I-PRF). A-PRF is created after centrifugation and coagulation, during which a membrane layer rich in growth factors, platelets and leukocytes is separated, which has a great ability to regenerate. It is used in tooth extraction, placement of implants, and completion of bone and soft tissue defects. I-PRF is a fraction that is in liquid form and is separated by centrifugation without anticoagulants, unlike A-PRF. The advantage of the I-PRF method is direct injection in the site where regeneration is needed. The liquid form is most often used in the field of aesthetic medicine in anti-aging, while in dentistry it is used to restore gums and increase soft tissue vascularization¹⁰. I-PRF is a second-generation, fully autologous blood-derived biomaterial that has a three-dimensional fibrin network, like a PRF clot, while maintaining a fluid nature, just like platelet-rich plasma (PRP). In addition to platelets and their growth factors, injectable PRF predominantly contains collagen type 1, lymphocytes together with their growth factors. This biomaterial is used in conditions where PRP and PRF clots are currently used, such as androgenetic alopecia, periorbital rejuvenation, temporary fillers, and as a means of promoting wound healing with favorable results¹².

The regeneration ability of PRF is owing to its angiogenesis potential, which can be explained by the 3D fibrin matrix that becomes biologically adhesive and enables stabilization of the initial platelet aggregation during coagulation. PRF can also recruit stem cells from circulating blood. Chouckroun *et al.* report on immune benefit of PRF¹³.

Materials and Methods

For research purposes, a prospective study was used. The study was approved by the institutional Ethics Committee, and all the participants provided informed consent. Data were obtained at the Department of Vascular Surgery, Merkur University Hospital, in the period from November 2021 to February 2023. In this pilot study of 16 observed patients, eight of them were bandaged with a combination of PRF and sponge dressing, and a control group of eight patients were bandaged with sponge and silver dressings. Perioperative parameters used in the study were gender, age, wound size, wound healing speed, postoperative infection, antibiotic prophylaxis, and extended amputation. Both sexes were represented, and steno-occlusive disease had been confirmed by the history, clinical examination, doppler, and CTA. All patients included in this study had steno-occlusive changes on the superficial femoral artery, the passage popliteal artery, and at least 2 of the 3 passage femoral arteries (anterior tibial artery, posterior tibial artery, fibular artery); after that, they underwent FP bridging with a polytetrafluoroethylene prosthesis. After surgery (bypass), on postoperative day 1, each patient underwent amputation of a part of an extremity (amputation of a toe, a small part of the foot, or transmetatarsal amputation), after which the wound was bandaged in the Department ward for 3 consecutive days with a

hypertonic solution. With patient consent, blood was taken from the radial or brachial artery, depending on the difficulty of access, at the Department of Vascular Surgery, Merkur University Hospital. The procedure of obtaining PRF is such that the patient's blood was placed in special tubes (Fig. 1a) and centrifuged for 8-10 minutes at 1300 rpm. A PRF Duo Quattro centrifuge was used in the centrifugation process (Fig. 1b).

The formation of the PRF membrane itself is very simple, that is, the basic education of a vascular surgeon is enough to work with the device for a few hours. In the PRF set with a centrifuge, there is the corresponding instrumentation required for the formation of the PRF membrane, such as special A-PRF+ vacuums, and the PRF Box for the formation of the PRF membrane. After centrifugation, a membrane in the form of a gel was formed (Fig. 1c), which was placed on the wound after previous debridement and washing with Granudacyn. A secondary non-adhesive spongy dressing is placed on the applied PRF gel, and a certain part of the lower extremity is bandaged with sterile gauze and a bandage. A secondary non-adhesive spongy dressing is placed on the applied PRF gel, and a certain part of the lower extremity is bandaged with sterile gauze and a bandage. Dressing was exchanged every other day and bypass patency was evaluated with doppler at each examination. Figures 2 and 3 show noticeable wound healing after amputation by weeks.



Fig. 1. (a) The process of forming PRF; (b) PRF Duo Quattro centrifuge; (c) obtaining the PRF membrane.



Fig. 2. Wound in (a) 1st week and (b) 2nd week after amputation.



Fig. 3. Wound in (a) 3rd week and (b) 4th week after amputation.

The statistical package Minitab19 was used on statistical analysis and processing of the data collected. The Anderson-Darling normality test showed how the collected data behaved relative to normal distribution, and parametric tests were selected to test the hypothesis. The t-test was used on comparison of the two groups of data.

Results

The number of patients observed in this pilot study was 16, mean age 72.6, of which 6 were men and 10 were women. Group 1 included 8 patients treated with standard dressings (primary silver and secondary sponge), of which there were an equal number of men and women, mean age 73.1. Group 2 of 8 respondents included 2 men and 6 women, mean age 72.1 (Table 2). A statistical t-test was performed in order to show that there was a statistically significant difference in the wound healing time between groups 1 and 2. The level of statistical significance was set at $\alpha=0.05$. Statistical hypotheses were set as follows:

$$H0: \mu_1 - \mu_2 \geq 0 \text{ (or } \mu_1 \geq \mu_2)$$

$$H1: \mu_1 - \mu_2 < 0 \text{ (ili } \mu_1 < \mu_2)$$

After conducting the test, a p-value of 0.000 was reached, which means that there was enough evidence to reject the null hypothesis. In other words, there was a statistically significant difference in the wound healing time between groups 1 and 2, i.e., the wound healing time was shorter in group 2 (PRF) than in group 1 (dressings only). Figure 4 shows arithmetic means of the wound healing time in both groups with the associated 95% confidence interval.

Furthermore, the existence of a statistically significant difference in the proportion of patients with an infection between groups 1 and 2 was analyzed using the χ^2 -test. The test resulted in $p=1.000$, which means that there was no statistically significant difference in the proportion of patients who developed an infection between groups 1 and 2. Also, the χ^2 -test showed that there was no statistically significant difference in the proportion of patients who used antibiotics between groups 1 and 2 ($p=0.285$). The next variable analyzed was extended amputation. The χ^2 -test yielded $p=0.028$. Since the p-value was lower than the given level of significance of the test, it was concluded that there was a statistically significant difference in the proportion of patients who underwent extended amputation between group 1 and group 2, i.e., it was statistically higher in group 1 (Table 3).

Table 2. Patient age and gender distribution

Total number of respondents		16	%
Mean age		72.6	
Men		6	38
Women		10	63
Group 1	Number of respondents	8	50
	Men	4	50
	Women	4	50
	Mean age	73.1	
Group 2	Number of respondents	8	50
	Men	2	25
	Women	6	75
	Mean age	72.1	

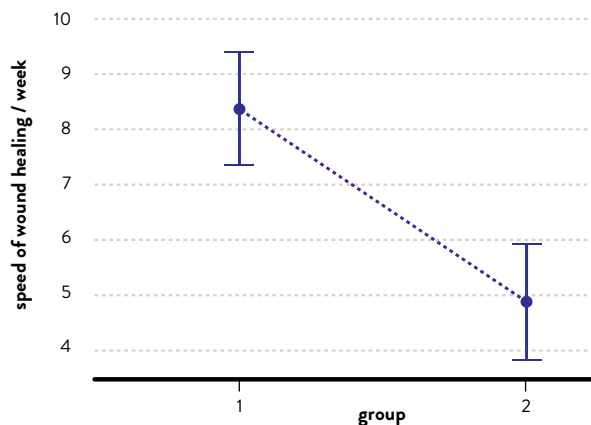


Fig. 4. Arithmetic mean and confidence interval of the performed t-test.

Table 3. Analysis of statistically significant difference between the groups

Variable	Test performed	P	Significant difference
Wound healing time	t	0.000	Yes
Infection	χ^2	1.000	No
Antibiotic	χ^2	0.285	No
Extended amputation	χ^2	0.028	Yes

The study showed that patients treated with dressings and PRF had faster wound healing than patients treated only with dressings.

Discussion

Considering the high percentage of extended amputation after revascularization and the parallel growing demand for more effective wound dressing methods, the idea of using PRF as an alternative method to standard wound dressings is being developed. In the database, we did not find studies that used this method in the pathology of vascular surgery. Therefore, our goal in this discussion is to show how treatment with PRF dressings in vascular surgery has positive effects, as well as in other branches of medicine⁸⁻¹⁰.

The use of fibrin-enriched plasma for topical application is of particular interest for promoting healing of the skin and mucosal wounds. Indeed, fibrin-based surgical adjuvants have been widely used in plastic surgery, dentistry and orthopedics for many years to improve scar healing and wound closure^{14,15}.

There are numerous works that show the positive effects of PRF. Using this method in dentistry has significantly reduced postoperative pain, especially in the first 3 days after tooth extraction. Also, soft tissue healing was significantly improved in the PRF group compared with spontaneous wound healing at 1 week (75% of studies evaluated). Another positive effect of the mentioned method is the loss of bone mass, which was significantly lower in the PRF group compared to spontaneous wound healing after 8-15 weeks (comparison with extended amputations). Likewise, in 85% of the studies, filling of the alveolar cup was significantly

higher in the PRF group compared to spontaneous wound healing. According to Al-Maawi *et al.*, PRF is most effective in the early period of wound healing, in 2-3 months after tooth extraction¹⁶. In our study, dressings were started a week after amputation. In the first week, only hypertonic solution was used, and the advantage of PRF over classic coverings has been statistically demonstrated.

Bao *et al.* confirmed with their results that PRF derivatives reduce only some postoperative symptoms, but do not prevent them all. Application of A-PRF after third molar extraction reduced postoperative pain, and L-PRF improved the degree of soft tissue healing¹⁷.

In general, PRP is mainly used in cases of hard and soft tissue procedures, while PRF is used in gingival recession and treatment of furcations and intrabony defects. In 2000, Choukroun *et al.* have reported a new alternative to PRP: PRF¹³. This biomaterial was developed for use in maxillofacial and oral surgery. The use of PRF differs from PRP because it does not require the use of thrombin or anticoagulants, but only centrifuged autologous blood. Moreover, PRF can also recruit stem cells from circulating blood. Choukroun *et al.* have reported an immune benefit of PRF. These advantages could explain why there are fewer postoperative infections when using this technique¹³.

Compared to the study by Alrayyes and Al-Jasser¹⁸, where the use of PRF showed significant results in alveolar bone healing outcomes, this pilot study shows similar wound healing results in vascular surgery. On the other hand, most studies included reports that mixing PRF with grafting material showed the best result, e.g., Miron *et al.* showed that the use of PRF in combination with soft tissue graft could represent a valid treatment modality for gingival recession¹⁹. A meta-analysis of the included studies proved the beneficial effect of PRF in alveolar cup preservation operations alone or in combination with other grafting materials¹⁸.

All studies investigating alveolar ridge soft tissue healing after PRF extraction showed better results in groups where PRF was added or used alone compared to natural treatments because it more successfully limited dimensional changes after extraction²⁰.

Furthermore, in aesthetic medicine, PRF is useful in wound healing and skin rejuvenation as a primary and supplementary technique due to its fibrin matrix,

cellular component and prolonged release of growth factors. The advantages are that PRF is easy to obtain, inexpensive and can be applied locally, injected or combined with other aesthetic procedures. In this regard, PRF has diverse and increasingly relevant capacities in aesthetic medicine and surgery. PRF is also used with mesotherapy to restore tone to the skin, which is an increasingly popular method, and infection is not possible given that it is one's own blood²¹. Lin and Sclafani propose that PRF can be considered a valid anti-aging treatment modality, i.e., for the purpose of rejuvenation²².

As this pilot study showed, this way of treating patients with the PRF method after revascularization showed good results; a statistically significant difference was obtained compared to the time of wound healing in patients who were treated only with standard dressings. The result of this study suggests a clear indication that this type of treatment in patients who have undergone revascularization and amputation of a limb should be introduced into the standard treatment procedure in vascular surgery.

Conclusions

Peripheral arterial disease (occlusion or stenosis of blood vessels in extremity) is one of the most common vascular diseases and its complications, such as chronic wound, pose a major public health and socioeconomic problem. Patients who undergo revascularization of lower extremities and who have a chronic wound, come to vascular department for weeks, months, and sometimes even years to repair and dress the wound.

In clinical practice, it happens in many cases that such patients end up with an extended level of amputation due to complications caused by insufficiently fast closure of a chronic wound, and sepsis can often develop due to wound infection.

Therefore, as shown by this pilot study, this treatment model could play a significant role in improving the treatment of chronic wounds after revascularization and contribute to the development of vascular surgery.

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Sažetak

ULOGA TROMBOCITIMA OBOGAĆENOG FIBRINA U BRZINI CIJELJENJA RANE NAKON REVASKULARIZACIJSKOG ZAHVATA

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Jedna od najčešćih bolesti u vaskularnoj kirurgiji je periferna arterijska bolest posljednji stadij koje je kritična ishemija ekstremiteta. Obilježava ju bol u mirovanju koji traje duže od 2 tjedna, odnosno gangrenozne promjene donjih ekstremiteta. Nakon učinjenih pretraga takve bolesnike često je potrebno liječiti otvorenim kirurškim, odnosno endovaskularnim pristupom. U većini slučajeva nakon revaskularizacije, a zbog gangrenoznih promjena, potrebno je učiniti amputacijski zahvat dijela donjeg ekstremiteta. U provođenju ovog probnog istraživanja dio bolesnika je previjan trombocitima obogaćenim fibrinom (*platelet rich fibrin*, PRF) u kombinaciji s oblogom, dok je drugi dio liječen samo oblogama. PRF membrana se formira metodom centrifugiranja. Svi promatrani bolesnici uključeni u ispitivanje potkoljenično su imali najmanje 2 od 3 prohodne krvne žile te im je ugrađeno femoropoplitealno premoštenje. Bolesnici iz ove studije praćeni su od studenoga 2021. do veljače 2023. godine prema parametrima brzine cijeljenja rane. Kod bolesnika čije su rane tretirane PRF-om nakon revaskularizacije prosječno razdoblje cijeljenja je iznosilo 5 tjedana, za razliku od 8,5 tjedana kod onih čije su rane previjane samo oblogama, a u nekih je bolesnika bilo neophodno i proširiti razinu amputacije. Nakon provedenog istraživanja donosi se zaključak da se ovakav način liječenja može na siguran način primijeniti u liječenju bolesti vaskularne patologije.

Ključne riječi: *Periferna arterijska bolest; Kritična ishemija ekstremiteta; Trombocitima obogaćen fibrin; Amputacija*