

Research Article

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The effect of smoking on posttraumatic pseudoarthrosis healing after internal stabilization, treated with platelet rich plasma (PRP)

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Abstract: Disturbed or delayed healing remains one of the most serious fracture-related complications, despite bone capacity for internal regeneration and reabsorption. Considerable progress in the understanding and treatment of fractures has been noted. The aim of our study was to evaluate treatment outcome in patients (smokers and non-smokers) with post-traumatic pseudoarthrosis.

Hypothesis: Determinate when administration of growth factors is most beneficial, and whether it accelerates bone union.

Material and methods: The study included patients treated for post-traumatic pseudoarthrosis resulting from multiple bone fractures. The study group and controls were further subdivided into: non-smokers, non-smokers >2 years after quitting, and smokers. Independent tests were performed for men and women. The study group, apart from other methods of treatment, received concentrated PRP (platelet-rich plasma) to aid the process of bone healing, or in cases of delayed healing confirmed by radiological assessment on follow-up visits.

Results: Mean time of fracture healing was 8 weeks for non-smokers and non-smokers >2 years after quitting,

whereas in smokers the healing process was significantly prolonged (18 weeks in both, men and women).

Conclusions: The risk for infection is smaller in non-smokers as compared to smokers, with the latter being at an elevated risk for bone inflammation and delayed union.

Keywords: Bone pseudoarthrosis; Bone defect; Platelet rich plasma

Level of evidence: III

1 Introduction

Despite medical advances treating pseudoarthrosis and disturbances of bone union, they remain one of the greatest challenges of modern orthopedics [1]. Restoration of proper blood flow by anatomical positioning of the fractures and application of pressure to minimize the fracture gap, well as the fastest possible mobilization, are the conditioning factors for fracture healing. Regardless, even adequate surgical technique and application of the best methods of osteosynthesis do not always result in proper fracture repair [2]. Thus, the search for new methods to accelerate the process of osteosynthesis continues [3].

Disturbed healing constitutes one of the most serious complications from fractures, despite bone capacity of internal regeneration and repair, progress and developments in their treatment, as well as understanding of the fracture process itself. Treatment methods which might aid the process of healing include, among others, the use of PRP (platelet-rich plasma). Healing may be accelerated by administering PRP that contains growth factors to the non-healing fracture area, thus improving bone and soft tissues, and boosting their regenerative potential [4].

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That method is often used due to the ease of preparation and acceptable price. PRP requires only a blood sample instead of more invasive procedures of bone marrow transplant. Blood platelets produce numerous growth factors and PRP is an autologous source of the platelets [4]. However, reports on the efficiency of using PRP often present conflicting results [5-8]. Adequate surgical technique, complete stabilization of the bone fracture, as well as patient lifestyle, determine proper bone healing.

Despite known threats related to smoking and continuous warnings, its prevalence is estimated at 1 billion people worldwide. Nowadays, it is known that certain diseases are directly related to tobacco use, e.g. lung diseases, ischemic heart disease, or increased tendency to bone fracture [9,10]. Most physicians are of the opinion that smoking significantly influences the process of wound healing, including bone union, even though that exact mechanism remains to be fully elucidated. Cigarette smoke disturbs bone metabolism, whereas its components increase bone resorption [11], and disturb osteoblast function [12]. The literature reports that smoking has a detrimental effect on fracture healing [13-15]. In light of the above, the question whether quitting smoking brings positive consequences remains unanswered, but most authors report long- and short-term benefits of smoking cessation [16, 17]. At present, the body of evidence indicating that patients with lower extremity fractures should stop smoking immediately. This is because it significantly improves bone healing and has a very low correlation with complications. The affected individuals should stop smoking at least 4 weeks before the procedure and continue throughout the entire rehabilitation process. These measures significantly reduce the costs of treatment due to shortened hospitalization, as well as a smaller number or reoperations in patients with pseudoarthrosis. Cost analysis revealed that the length and cost of hospitalization may be two-times lower if PRP has been administered.

The aim of our study was to evaluate the treatment outcome in patients with pseudoarthrosis and to determine when administration of growth factors is most beneficial, and whether it accelerates bone union.

2 Material

The study was conducted at the Department of Orthopedics, Traumatology and Oncology of the Locomotor System, Pomeranian Medical University in Szczecin, between 2007-2014. The study has been adhered to the

tenets of the Declaration of Helsinki. Ethics Committee ruled that approval was not required for this study.

A total of 200 patients, divided into two equal groups, constituted the study group and controls. All subjects were treated due to post-traumatic pseudoarthrosis of the tibia as a result of multiple bone fracture. The study and the control groups were further divided into three subgroups: non-smokers (28 – study group, 48 – controls), non-smokers >2 years after quitting (16 – study group, 12 – controls), and smokers (56 – study group, 40 – controls). Also, gender-based division was introduced.

The control group was treated surgically and the choice of the method was based on the type of pseudoarthrosis. Stabilization with reamed intramedullary (IM) nails, unreamed IM nails, or stable LCP plate fixation was used (35, 10, and 55 patients). Unreamed IM nails were used for pseudoarthrosis cases with enhance periosteal reaction, whereas drilling was used if little reparative periosteal reaction was observed. Resection of the pseudoarthrosis and fixation with plate was used in patients after failed primary repair with IM nails. Bone marrow graft, harvested from the iliac crest, was used in patients with stable LCP plate fixation. Apart from the above mentioned methods, PRP was additionally used in the study group to aid bone healing or in cases of unsatisfactory bone healing confirmed on radiological follow-up. The time after which the patient received PRP was up to 3 months after pseudoarthrosis diagnosis.

All subjects were deemed eligible for surgery at the SPSK1 Orthopedic Out-Patient Clinic in Szczecin. The commonly accepted criteria of pseudoarthrosis, i.e. lack of progression of fracture healing for 3 consecutive months, and nonunion confirmed on clinical and radiological follow-up at 6 months since the primary treatment, were applied.

The diagnosis was based on confirmed pathological mobility of the bone fractures on clinical and radiological check-up. Typically, classic PA and lateral X-ray is sufficient, but in some cases MRI or bone scintigraphy were necessary.

All patients reported for check-up every 6 weeks. X-rays were performed on day 1 or 2, at 6 weeks, and at 3 and 6 months postoperatively. Fracture healing was evaluated on the basis of clinical evaluation, patient complaints, and assessment of the following: skin temperature, local swelling, pathological mobility of the bone fractures, as well as radiological tests.

Additionally, mean time to fracture healing with (study group) and without (controls) PRP administration, as well as incidence of unsuccessfully treated fractures (complete nonunion) after 36 months, infection at post-

operative wound site, and osteomyelitis were evaluated. Postoperative follow-up ranged from 6 months to 7 years.

2.1 PRP preparation (APC-60)

The APC-60 pack (Harvest) was used for patients who received PRP. Blood had been drawn preoperatively before any drugs were administered. Next, 6 ml of anticoagulant were drawn into 60 ml syringe and 54 ml of patient blood were added. The syringe was gently rotated and inverted a few times to mix the anticoagulant with the blood. Next, the blood with anticoagulant was injected through the red port into a special container, which was then placed in the centrifuge with counterbalance marked as APC-60 and centrifuged for 15 min. After completion, the vial was gently removed to avoid shaking and tilting. Next, excess of platelet-poor plasma was removed through the white port using a syringe with a needle and a spacer. Using a second syringe, without the spacer, the concentrate was drawn and injected a few times into the container chamber to accurately mix concentrate fraction and finally the entire content of the chamber was drawn into syringes and transferred to the sterile field to administer intraoperatively. The PRP concentrate was used at the very end of the surgical procedure, directly before suturing subcutaneous tissue and fascia. The wound was injected with the remaining platelet-poor plasma.

3 Results

3.1 Control group

The control group included patients after fracture who did not receive PRP. Mean time of healing for all non-smokers and non-smokers >2 years after quitting was 8 weeks (Table 1).

All these patients were deemed 'completely cured' until week 36. Postoperative wound infection was found in only 1 female patient from that group.

As far as smoking controls were concerned, mean time of wound healing after fracture was 10 weeks in both, men and women. What is more, 5 men and 3 women were deemed 'incompletely cured' until week 36 of the observation. Also, postoperative wound infection was found in 5 smokers (2 men and 3 women), and osteomyelitis was observed in 3 men.

3.2 Study group

The study group included fracture patients with healing disturbances, treated with PRP. Mean time of healing for non-smokers was longer: 12 weeks for men and 10 weeks for women (Table 1). Incomplete union until weeks 36 of treatment was found in 2 men and 3 women who never smoked and 1 person from group of non-smokers >2 years after quitting. In that group of patients, postoperative wound infection was found in 1 man and 1 woman, and osteomyelitis was observed in 1 patient.

Significantly prolonged mean healing time was observed among smokers: 18 weeks in both, men and women. Incomplete healing was diagnosed in as many as 12 (34%) men and 8 (38%) women until postoperative week 36. In the group of smokers, postoperative wound infection was found in 1 man and 3 women, whereas osteomyelitis was observed in 16 patients (11 men and 5 women).

4 Discussion

Initial clinical applications of platelet concentrates that contain growth factors was limited to oral and maxillofacial surgery. The effectiveness of platelet concentrates in bone regeneration has been proven in dental implants [18], thus they have found their way into orthopedic surgery [19].

Improved bone union and beneficial effects on bone regeneration after administration of platelet concentrates that contain growth factors are the reasons why researchers look for other areas to apply platelet concentrates than just oral and maxillofacial surgery. Franchini et al. [20], used platelet concentrates in fractures, pseudoarthrosis, bone reconstruction, hip joint replacement surgeries, as well as in fibrous dysplasia and bone inflammation. Bibbo et al. [21], achieved good results in high-risk patients undergoing arthrodesis of the ankle joint, thus signaling the possibility of applying platelet concentrates in new fields.

The attempt to improve integration of the anterior cruciate ligament replacements in bone tunnels for knee joint replacement surgery is yet another use of platelet concentrates. Preliminary clinical reports seem to indicate improved transformation and integration of the graft. In a study by Ventura et al. [22], CT data demonstrated a faster transformation of the graft, from autologous into a ligament structure, in PRP patients and controls. Regardless, the clinical and subjective data were the same in both

Table 1: Effect of smoking on healing of fractures in men and women treated with platelet rich plasma (PRP)

Smoking status among patients	Average fracture healing time without PRP and since PRP administration [weeks]				Uncured until the 36 th week (%)				Postoperative wound infections (%)				Osteomyelitis (%)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Non smoker	Males	30 (100%)	13 (100%)	8	12	0	2 patients (15%)	0	1 patient (0,7%)	0	0	1 patient (0,7%)	0	1 patient (0,7%)
	Females	18 (100%)	15 (100%)	8	10	0	3 patients (33%)	1 patient (2%)	1 patient (0,7%)	0	0	0	0	0
Non-smoker (>2 years after quitting)	Males	7 (100%)	6 (100%)	8	12	0	1 patient (17%)	0	0	0	0	0	0	0
	Females	5 (100%)	10 (100%)	8	1	0	1 patient (10%)	0	0	0	0	0	0	0
Smoker	Males	22 (100%)	35 (100%)	10	18	5 patients (23%)	12 patients (34%)	2 patients (9%)	1 patient (3%)	2 patients (9%)	3 patients (13,6%)	11 patients (31%)	3 patients (13,6%)	11 patients (31%)
	Females	18 (100%)	21 (100%)	10	18	3 patients (17%)	8 patients (38%)	3 patients (17%)	3 patients (14%)	3 patients (17%)	0	5 patients (24%)	0	5 patients (24%)

groups. However, these authors observed a synovitic reaction in 1 of the 10 patients from the group which received platelet concentrate.

On the other hand, Sanchez et al. [23], emphasize lower incidence of inflammatory reaction after reconstruction if platelet concentrates were used. The question whether it was caused by different leukocyte concentration in their platelet concentrates containing growth factors remains to be elucidated.

Yamada et al. [24], evaluated the effectiveness of treating bone defects with the use of PRP combined with mesenchymal stem cells (MSCs). In animals, these authors observed newly formed bones at the site of bone defect after 2 weeks, and proper structure of both, the cortical layer and the trabecular bone after 8 weeks. In our study, we noted similar results in non-smoking controls. The results of Yamada et al. [24], were more significant than if only platelet concentrate containing growth factors was used, or if the defect was left unfilled. Interestingly, their results were also better than if the defect was filled with autologous bone marrow transplants, as in that case resorption foci at the site of the transplant were observed after 8 weeks, whereas such effect was not reported after using PRP.

Lucarelli et al. [5], investigated the effect of PRP on proliferation of stromal stem cells in humans and observed cell growth on days 3, 6 and 9 of the experiment. On day 6, they noted slightly increased proliferation of the stromal cells after using 1% PRP but treatment with 10% PRP induced marked cell proliferation (over 2-fold). They concluded that these results suggest a possibility of treating large bone defects with the use of stromal stem cells mixed with PRP.

Similar findings were published by Romin et al. [6], who investigated in vitro proliferation of bone marrow cells mixed with PRP, cultured on ceramic materials with osteoconductive properties. In the PRP group, proliferation of bone marrow cells was enhanced (by 31%), and the alkaline phosphatase activity also increased (by 31%) after 15 days culture.

Compromised integrity of bone tissue triggers a number of natural processes of regeneration, thus ensuring speedy reconstruction of its integrity and function. However, in some cases the process may be disrupted, resulting in the formation of pseudoarthrosis and creating a need for surgical re-intervention.

Current state of knowledge on bone tissue regeneration allows to accelerate and modify the process by using transplants with PRP containing growth factors. Blood platelets represent a reservoir of growth factors in a human body, playing various important functions in

the process of coagulation [25-27], immune response, and healing of injured tissues [28, 29].

Bone regeneration is initiated when PDGF, TGF- β 1 and IGF are released from the granules of platelets in the transplant. PDGF stimulates mitosis of the bone marrow stem cells and osteoblasts from the transplant, resulting in their increase by several orders of magnitude. Moreover, PDGF initiates angiogenesis and vascular integration into the graft by increasing the mitosis of endothelial cells. TGF- β 1 stimulates fibroblast and pre-osteoblast proliferation, thus increasing their number and promoting their differentiation to mature osteoblasts. TGF- β 1 influences osteoblasts which lead to the formation of osteoid. Fibroblasts, stimulated by TGF- β 1, synthesize extracellular matrix and support integration of small vessels. First blood vessels may be observed already on day 3 after regeneration was initiated, whereas completion of capillary penetration occurs on days 14–17 [30]. Insulin-like growth factor (IGF) affects osteoblasts inside the bone, which reinforce trabeculae osseae within the trabecular bone graft. The initial intensification of cell activity is the direct result of PDGF, TGF- β 1 and IGF action, and to a lesser extent other growth factors [30].

Reports on PRP application in orthopedics remain limited. Lowery et al. [31], used PRP and autologous growth factor concentrate (AGF) in lumbar spinal fusion and achieved very good results. These authors observed no radiological or clinical features of pseudoarthrosis in any of their patients and achieved bone union. Kitoh et al. [32], used PRP and marrow-derived mesenchymal stem cells (MSCs) in distraction osteogenesis in 3 patients and noted accelerated bone regeneration between days 34 and 47. It forced them to increase the distraction rate to 1.5 mm/day. Accelerated bone regeneration was observed already after first administration of PRP and MSCs. These authors admit that, in light of the fact that two osteoinductive materials were combined, it is difficult to draw unambiguous conclusion about PRP effect on bone formation.

Since the literature offers only a limited number of reports on clinical application of PRP in treating disturbed bone union, and in light of highly promising results of bone regeneration in the field of oral and maxillofacial surgery, researchers are forced to continue with their efforts to discover and establish recommendations on gel application in clinical practice [33].

Percutaneous PRP injection into the nonunion gap has been performed. In some patients, minimally invasive technique is often enough to achieve union. However, no effect of percutaneous PRP injection on the healing process was noted in approximately 50% of patients from the entire group with pseudoarthrosis. However, union

was obtained in all patients with nonunion when PRP was combined with autologous bone graft [33].

In patients with fractures, smoking constitutes a serious risk factor for prolonged union time, as well as for the development of additional complications. The exact mechanism of how smoking handicaps fracture healing remains unclear. The underlying mechanisms include the hypothesis about decreased blood flow caused by narrow vessels and impaired angiogenesis [34,35], oxygen deficiency, high level of reactive oxygen species, the effect of nicotine on endothelial receptors, or decreased collagen synthesis [36]. The effect is probably multifactorial, but it is not certain whether it is nicotine or other components of tobacco smoke that are responsible for the adverse changes.

The literature offers some reports that smokers are at a higher risk for nonunion [14,16,37]. Adams *et al.* [38], compared complication rates in 140 smokers and 133 non-smokers with open tibial fracture. Both groups were comparable in terms of demographics and primary treatment. Bone transplant was necessary in 26% of smokers as compared to 18% of non-smokers. Kyro *et al.* [39], investigated 135 patients with non-surgically treated tibial fractures. They determined that mean time to bone union was significantly longer in smokers as compared to non-smokers (166 vs. 134 days).

Although it remains unclear whether modification of smoking habits [40] influences fractures, it seems prudent to recommend cessation of smoking in smokers, at least during the time of bone healing.

Also, it cannot be excluded that adverse effects of smoking on bone healing are dose-dependent. We assumed, similarly to other researchers [14,16,37], that smokers may be defined as individuals who admitted to smoking at least 10 cigarettes a day.

Homogeneous sample is a definite strength of our study. Only patients with isolated unilateral fractures, with no serious soft tissue injury, were included. The same procedures of trauma management were used for all patients, regardless of their social background and gender.

A study by Moghaddam *et al.* [16], revealed that current and previous smokers are at an elevated risk for delayed union or nonunion of the body of tibia as compared to non-smokers.

Detrimental effect of smoking on fracture healing has been reported in the literature [14,38,39,41]. A prospective, multi-center study by Castillo *et al.* [14], documented an increased incidence of postoperative complications in smokers and ex-smokers. Taking into account the changes, prolonged time to complete healing was

observed in smokers and ex-smokers (by 37% and 32%, respectively) as compared to non-smokers.

Noteworthy, although ex-smokers are at higher risk for delayed union and accompanying complications, the risk is still smaller than in current smokers. Thus, it seems vital for doctors to encourage smoking cessation in their patients.

Smoking is one of the main factors that have a detrimental effect on the skeletal and muscular system. It has a negative impact on bone mineral density, degenerative changes of the intervertebral disc, incidence of femoral column fractures, and the dynamics of wound healing. That is why Sloan *et al.* [12], demonstrate that smokers are at a disadvantage at the very beginning of the therapy, and that treatment time will be significantly prolonged in their case. These authors encourage patients to stop smoking before surgical treatment. Castillo *et al.* [14], observed that smoking increases the risk for all complications, with infection and osteomyelitis as the two main threats. Also, prognosis for complete fracture healing is significantly worse in case of smokers. The risk for infection is smaller for ex-smokers as compared to smokers, but the risk for bone inflammation and delayed union remains greater than in non-smokers.

5 Conclusions

The risk for infection is smaller in non-smokers as compared to smokers, with the latter being at an elevated risk for bone inflammation and delayed union.

Conflict of interest: All authors declare that they have no conflict of interest. All authors read and approved the final manuscript.

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