Is Platelet-Rich Plasma Injection an Effective Choice in Cases of Non-Union?

Je injekční aplikace PRP účinnou možností léčby v případech pakloubu?

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ABSTRACT

PURPOSE OF THE STUDY

By the expression of several growth factors from activated thrombocytes, the application of Platelet Rich Plasma (PRP) stimulates angiogenesis and regeneration thus stimulating recovery through cell differentiation. This study aimed to evaluate the effects of PRP injection on patients who had undergone surgery for fracture and in whom delayed union or non-union had been determined.

MATERIAL AND METHODS

The study comprised 20 patients (male 17, female 3; median age 33.5 range 15–77) who had undergone lower extremity fracture surgery and were diagnosed with aseptic delayed union (8 patients) or non-union (12 patients). Blood taken from the patients was centrifuged to separate PRP, which was then activated by calcium chloride. The prepared PRP was injected into the fracture line under fluoroscopy guidance for totally three times once a week. The application of PRP was made at median 6 (range 6–8) months after fracture surgery. All patients were followed-up with clinical examinations and radiographs over a median period of 11 (range 8–12) months.

RESULTS

Fracture union was achieved in six patients at median 15 (range 8–24) weeks. There was non-union of the fracture in eleven patients during the follow-up period and these patients underwent revision surgery. Sufficient union was not determined radiologically and clinically in three patients. Fracture union was achieved in six of eight patients in the delayed union group. There was no patient in the non-union group with fracture union.

CONCLUSIONS

Fracture healing is a process affected by many factors. Although PRP has been reported in literature to be a biological treatment which increases healing, adequate healing was not determined in the treatment of non-union with PRP injection. However, in selected patients determined with delayed union, PRP injection can be recommended in non-surgical treatment.

Key words: platelet-rich plasma; delayed union; non-union; growth factors.

This study was presented as poster presentation in the Osteosynthese International Congress 14–17 February 2013 Çeşme-İzmir, Turkey
The abstract was published in Injury, Volume 44, Supplement 2, February 2013, Pages S43-S44

INTRODUCTION

Fracture healing is a complex physiological process involving a coordinated interaction of hematopoietic and immune cells within the bone marrow, in conjunction with vascular and skeletal cell precursors (32). Several different cytokines and growth factors are effective at different stages of this process (29). Despite this well-organized regenerative response, healing problems are seen in approximately 10% of fractures (8). A delayed union is an ununited fracture that continues to show progress toward healing or that has not been present for long enough to satisfy an arbitrary time standard for union (22). Non-union is defined by the United States Food and Drug Administration as established when a minimum of nine months has elapsed since injury and the fracture shows no visible progressive signs of healing for three months (15). Non-union is generally classified as hypertrophic, oligotrophic or atrophic according to the radiological appearance (15). Atrophic non-union is characterised by little or no callus and resorption in the bone ends. Normal healing is limited due to an insufficient biological reponse in the fracture line. In oligotrophic and hypertrophic non-union, blood flow is sufficient and an excessive amount of callus is seen. Insufficient mechanical stability is a reason which leads to non-union (28). In the treatment of delayed union or non-union, the biological and mechanical factors should be evaluated first. After achieving mechanical stability with internal or external fixation, an attempt is made to achieve union with grafts, growth factors or with physical means (electric, ultrasound, distraction osteogenesis) (22).

Platelet Rich Plasma (PRP) is a treatment form stimulating natural healing steps through growth factors contained in the platelets. PRP applied to the wound area accelerates the physiological healing process, provides support for the connection of cells, reduces pain and has an anti-inflammatory and anti-bacterial effect (26). Obtaining PRP growth factor is a simple, cheap and easy way (1). As it is autogenous in origin, easy to prepare and has an excellent reliability profile, it has opened the door to new treatment (16).

Studies in literature have reported the use of PRP in the treatment of non-union (4, 6, 25, 27). There are 16 concentration systems which can be used to obtain PRP. Leukocytes and growth factor contained in PRP are obtained in different amounts from these systems (7). Apart from the concentration systems, PRP can be obtained manually from peripheral blood (2, 3). Unanswered questions remain related to the application of PRP, such as the ideal volume, application frequency, application period and platelet activation (18).

The aim of this study was to determine the effects on union of PRP obtained manually as a cheap and easy method in the treatment of patients diagnosed with delayed union or non-union. The hypothesis was that to obtain union with percutaneous injection of manually-prepared PRP.

MATERIAL AND METHODS

This study was a prospective case series. Approval was granted by the Local Ethics Committee and informed consent was obtained from all patients participating in the study. The study comprised patients who had undergone surgery for a lower extremity fracture and at the six month postoperative clinical examination had pain on weight-bearing, sensitivity and movement determined in the fracture line or who had no findings of progression of union determined radiologically in three consecutive months. Exclusion criteria were upper extremity fractures, pathologic fractures, pregnancy, active tumour or haematological malignant disease, clinical or laboratory signs of infection, a history of anticoagulant use, Hb value < 11 g/dl, thrombocyte count < 150,000 mm^3.

A total of 23 patients who met the study criteria were enrolled in the study. However, three patients who discontinued follow-up were not included for evaluation. According to postoperative duration and direct radiograph findings, the patients were separated into two groups of delayed union and non-union by an experienced orthopedist who was blinded and not participating in the study. The delayed union group comprised eight patients and the non-union group comprised twelve patients.

The twenty patients included for evaluation were 17 males and 3 females with a median age of 33.5 years (range 15–77 years). Fourteen patients were operated on for a femoral fracture and six patients for a tibial fracture. Intramedullary nailing was applied to eleven patients,

Table 1. Comparison of patients data and results of both groups

	Delayed union group n=8	**Non-union group n=12**	**P value**
Age (year)	30 (18–77)	35 (15–51)	0.908*
Male/Female	6/2	11/1	0.306**
Time from first surgery (month)	6.5 (6–8)	6 (6–7)	
Follow-up (month)	11 (10–12)	10.5 (8–11)	
Union			0.001**
Yes	6	0	
No	1	10	
Not observed	1	2	

**Mann-Whitney test*
***Chi-square test*

plate-screw to five patients and external fixator to four patients.

Risk factors for non-union were determined as open fracture in two patients according to Gustilo-Anderson classification Type 3, open fractures in one patient according to Gustilo-Anderson classification Type 1, smoking cigarette in six patients and diabetes mellitus in one patient.

The preparation and application of PRP was made to all the patients under the same conditions. The method described by Anitua was used (2, 3). A total of 30 cc peripheral blood was taken from antecubital region of the patients into tubes containing 3.2% sodium citrate. The tubes were centrifuges at 1800 rpm for eight minutes at room temperature. From the 3.5 ml PRP which was obtained, 1 ml was sent to the laboratory for bacteriological test and platelet count. After activation, the 2.5 ml PRP containing 5.5% calcium chloride ($CaCl_2$) (50 µl $CaCl_2$ in 1 ml PRP) was administered to the non-union line under operating theatre conditions with flouroscopic control (Figs 1 and 3). A total of three doses of PRP were applied to the patients at intervals of one week. The result of the laboratory evaluation of the obtained PRP determined that the platelet count per mililitre increased by 400% compared to the thrombocyte count.

Fig. 1. Manually obtained PRP.

After the final application the patients were evaluated clinically and radiologically at 3-week intervals. Union was evaluated as healing in three of four cortices seen radiologically (34).

Statistical evaluation

Results were stated as median (minimum-maximum). Data were evaluated using SPSS software program (Windows Version 16.0). In the statistical evaluation of median values between groups, Chi-square test and MannWhitney U-test were used. A value of $P < 0.05$ was accepted as statistically significant.

RESULTS

No complications were seen in any patient during the applications or in the follow-up period. PRP was applied at median 6 months (range 6–8 months) after the first operation. The median follow-up period was 11 months (range 8–12 months) (Table 1).

The fractures of six patients achieved union at median 15 weeks (range 8–24 weeks). There was non-union of the fractures of 11 patients; grafting and revision surgery was applied to these patients. In three patients, sufficient union clinically and radiologically was not achieved at the end of 12 months follow-up.

In the delayed union group, union was achieved in six of eight patients. Revision surgery was applied to one patient where insufficient union was determined. Insufficient union clinically and radiologically was determined in one patient at the end of the follow-up period.

In the non-union group, revision surgery and grafting was applied to 10 of 12 patients where insufficient union was determined. At the end of the follow-up period, insufficient union clinically and radiologically was determined in two patients.

DISCUSSION

There are several factors which affect fracture healing. These can be grouped as factors related to the fracture (open fracture, infection, type of fracture), factors related to the patient (smoking, diabetes mellitus, connective tissue diseases, systemic infection) and iatrogenic factors (medications such as non-steroid anti-inflammatories and steroids) (30). In the treatment of non-union, autologous bone grafting is the first choice. Cancellous grafts taken from the iliac wing in particular have osteogenic, osteoinductive and osteoconductive properties. They are widely used as the gold standard compared to other grafts (22, 28). Alternative treatments to autologous grafts should have low morbidity and be as effective as autografting (35).

Although autologous bone grafting has several advantages, it may lead to complications such as infection, haematoma, seroma, postoperative pain, and loss of sensation. Two separate studies have reported major complication rates of 2.4% and 8.6% (10, 36). As an alternative to autologous bone grafting, studies have reported the use of bone marrow injection, bone morphogenetic protein (BMP), PRP, and demineralised bone matrix (4, 5, 6, 24, 25, 27, 35).

PRP was first used in 1987 in heart surgery to prevent excessive blood transfusion (9). More than 30 bioactive proteins are found within the alpha granules of platelets (2). Transforming Growth Factor beta (TGFβ) and Platelet Derived Growth Factor (PDGF) are growth factors with a major role in fracture healing (29, 32). PRP, which includes growth factors such as primarily TGFβ and PDGF, vascular endothelial growth factor, and insulin-like growth factor and proteins such as fibrin, fibronectin, vitronectin and thrombospondin plays a role at several stages of tissue healing (1). The growth factors activate some of the cells which have a function in tissue healing and thus provide soft tissue healing and bone regeneration (17). PRP activates repair cells in the blood circulation (13). With the effect of growth factors which it

contains, it stimulates local stem cells and activates the repair cells in the circulation and the bone marrow.

In a study by Bielecki et al. (4) of a single dose application of PRP to 32 cases of delayed and non-union, results were reported of union in all the delayed union group and in 65% of the non-union group. They recommended the application of PRP within 11 months after surgery. Sanchez et al. (25) applied revision surgery, grafting and PRP to 13 of 16 cases of non-hypertrophic non-union and repeated PRP injections to three patients. Union was obtained in all cases and at least three injections were recommended in cases where injection was applied. In a study by Calori et al. (6) in which revision surgery together with PRP was compared with the application of BMP7. In 120 cases of atrophic non-union, union was achieved in 86.7% of the BMP group and 68.3% of the PRP group and clinical and radiological healing was reported earlier in the BMP group. Griffin et al. (12) reviewed the use of PRP in clinical studies and reported that PRP use was safe but no clinical evidence was shown of benefit in acute or delayed fracture union. In the current study, union was determined in 30% of patients with PRP application. When evaluation was made according to the groups, although union was determined in 75% of the delayed union group patients, union was not determined in any of the non-union group. In the current study, three doses of PRP were administered at one per week as recommended in literature and at median 6 months (range 6–8 months) after the first surgery.

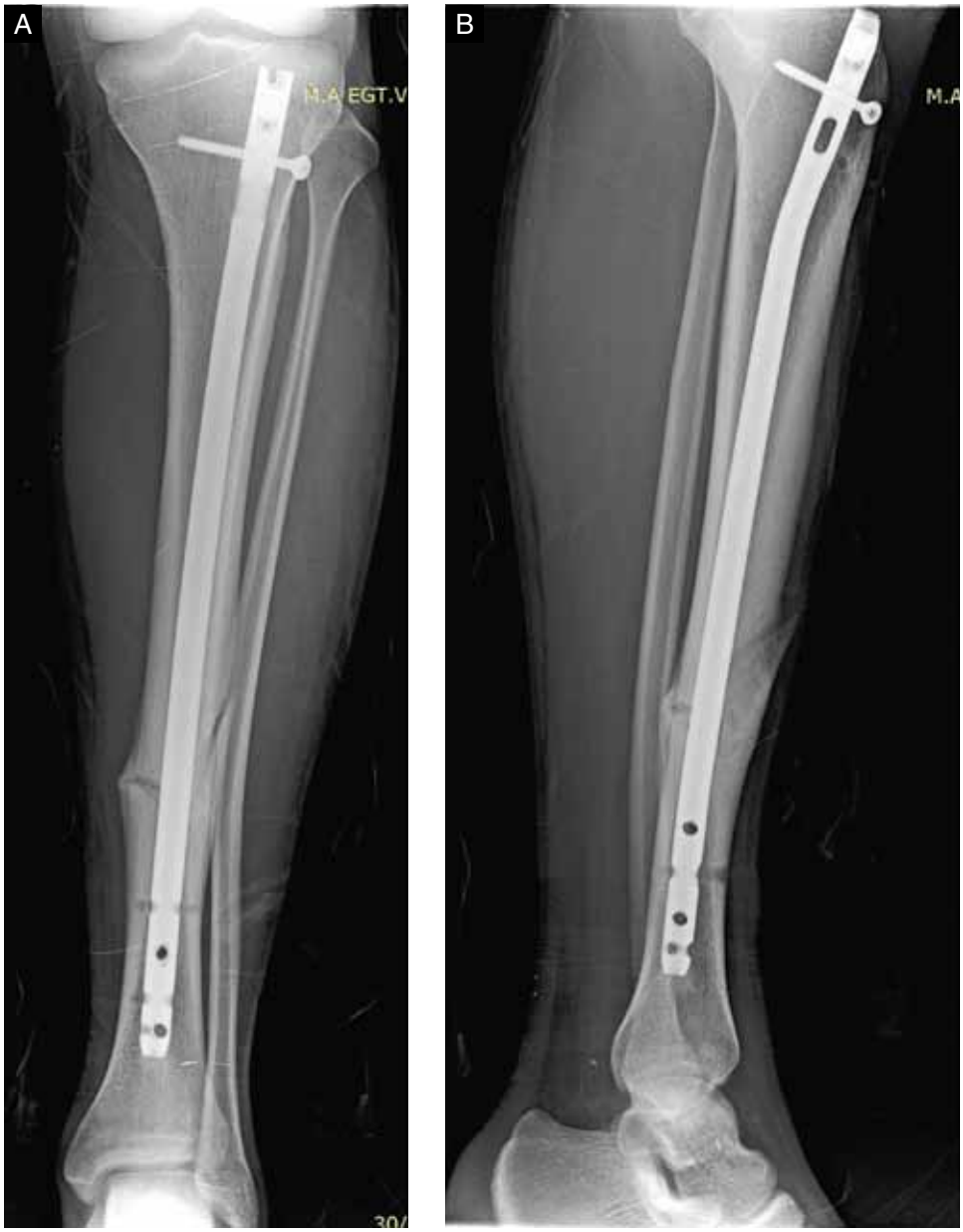

Fig. 2a-b. Anteroposterior and lateral radiograph of the 18-years-old patient who was diagnosed with delayed union after six months later from intramedullary nailing. Distal screw of the nails were removed for dynamization two months ago.

In histomorphometric studies which have examined the effects of PRP on bone healing in the short and long-term, a greater positive effect of PRP on bone healing has been reported in the short-term (3 months) compared to the long-term (6 months) (31). In atrophic non-union, there is something lacking in the biological process (15, 22, 28). Living bone cells are required for PRP to be able to be effective (24). That union was not achieved in the non-union group with PRP injection in the current study, is thought to be associated with there not being any living bone cells in the non-union fracture line and that PRP was administered a late stage.

Different results have been reported from the application of PRP together with allograft or autograft in surgical treatment. While some researchers have maintained that PRP has positive effects (14, 33), others have claimed that there is no benefit (20, 21).

Three different methods can be used to obtain PRP; automatic machines and commercial kits with double spin rotation, single spin rotation and manual PRP se-

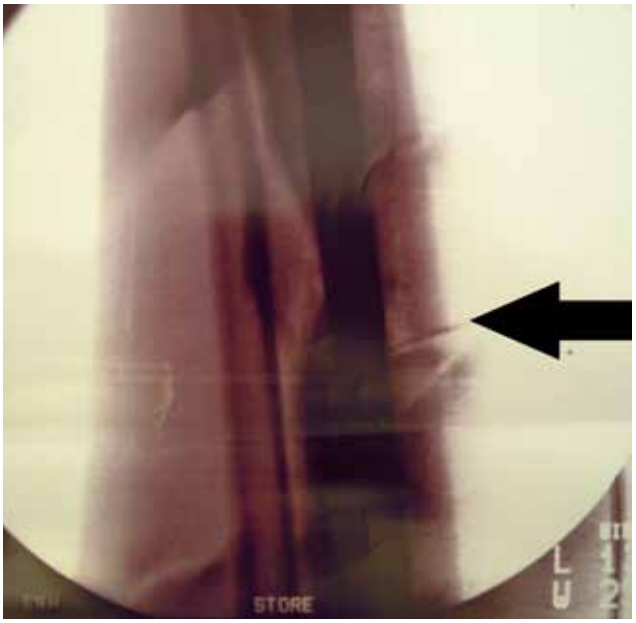

Fig. 3. PRP administation with flouroscopic guidence. Black arrow shows needle.

paration and selective blood filtration (plateletpheresis). Anitua reported that a platelet count over 300,000/µl in PRP is effective (3). In another in vitro study, platelet concentration 2.5 times greater than the basal platelet count was reported to be the most effective (11). The prepared PRP is activated by adding bovine or human thrombin or calcium chloride (23). Growth factors and cytokines are revealed with the formation of platelet gel from the activated PRP. In the current study PRP was prepared as single spin rotation and manually. In the analysis of the prepared PRP, concentration was determined as four times greater than the thrombocyte count in the peripheral blood. The prepared PRP was activated by the additon of calcium chloride.

The manual method of obtaining PRP used in the current study is low-cost and effective. While the cost of automatic devices and kits to obtain PRP is several hundreds of dollars, the cost of the manual method used to prepare PRP was approximately ten dollars (19).

For PRP obtained from autologous blood, there is no risk of immune reaction or disease transfer. There are no studies in literature warning of hyperplasia, carcinogenisis or tumour growth of PRP (26). No complications were encountered in any patient in the PRP group of the current study.

The limitations of this study are that there was no placebo control group, there were no radiological and biological results during follow-up to be evaluated with clinical examination and radiologically, the number of patients was low and the follow-up period was short.

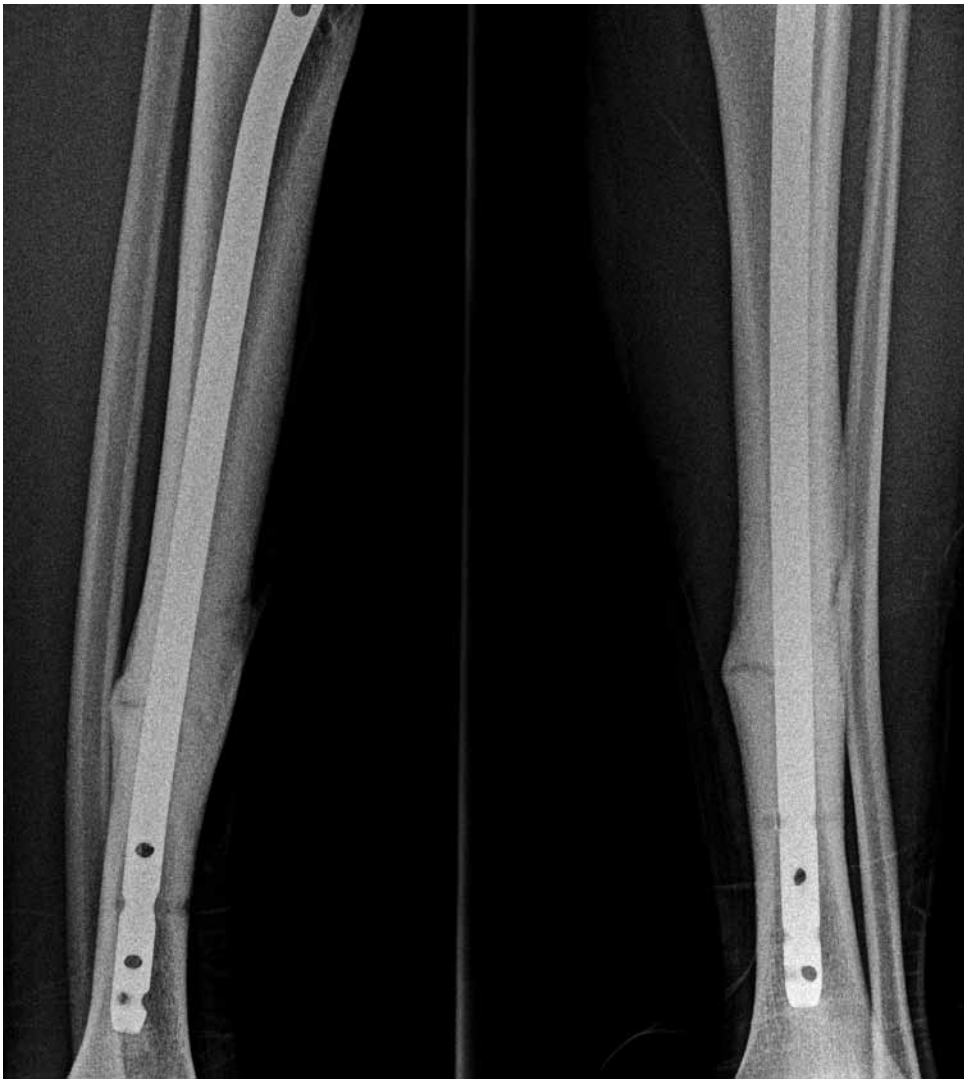

Fig. 4. Anteroposterior and lateral radiograph of the patient two months later. Union was achieved.

CONCLUSION

In conclusion, in the current study, sufficient union was not determined in the treatment of non-union with PRP injection. However, in selected patients determined with delayed union, PRP injection can be recommended in non-surgical treatment. Prospective, randomised, placebo-controlled, multi-centre studies are required to clarify these results and better understand the effects of PRP.

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