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ORIGINAL

Autologous platelet gel for delayed union of tibial shaft treated with intramedullary nailing

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Abstract Early recognition of a potential nonunion followed by early intervention reduces the ultimate time to union, and lessens the frustration for both patient and surgeon. The aim of this study was to evaluate the efficacy of treatment with autologous platelet gel (PG), rich in growth factors, in the treatment of delayed unions of tibial shaft fractures treated with locked intramedullary nailing. We treated 15 atrophic delayed unions of unstable tibial shaft fractures, stabilized with locked intramedullary nail. The mean time between the acute treatment of the fracture and PG application was 151 days. PG was injected into the site of unhealed fracture during closed surgery. The planned treatment programme was 3 radio-guided applications of PG at intervals of 3–4 weeks. Clinical and radiological evaluations 2, 4 and 6 months after the first application of PG showed gradual improvement of the clinical picture and progressive formation of bone callus. In almost all cases there were clinical and radiological improvements and it was possible to remove the synthesis aid an average of 12 months after the start of the therapeutic programme.

Key words Delayed union • Tibia • Platelet gel • Nailing

Introduction

Nonunion of the tibial shaft is a common problem, frequently leading to disability. Treatment may require multiple operations, prolonged hospitalization, and years of disability before union is obtained. Most tibial fractures heal after the initial treatment, but nonunion is seen by all practitioners who treat tibial fractures. Early recognition of a potential nonunion followed by early intervention reduces the ultimate time to union and lessens the frustration for both patient and surgeon [1–3]. Despite the numerous recent improvements in noninvasive treatment of nonunion, this condition is still a feared complication of fractures and a topic of discussion in the international scientific community. Although many dissenting options have been expressed on this case, delayed union is considered an ununited fracture that continues to show progress toward healing, or a fracture that has not been attended to enough to satisfy an arbitrary time standard for nonunion. Several authors used a settled period of time, ranging from 5 to 6 months, to define delayed union [4–6]. This has been confirmed by radiographs showing a lack of union at various time points.

Although the lack of precision in the definition diminishes its value in published reports, delayed union might best be thought of as the point at which one should consider a treatment to achieve union. Delayed union of a fracture occurs when the normal biologic healing processes of bone end, so that solid healing will not be achieved without further treatment [3].

Nonunions are classified according to their radiographic appearance as hypertrophic, oligotrophic, or atrophic as described by LaVelle [6]. This classification

helps to understand the mechanical and biologic factors contributing to the causes of nonunion, and can be used to direct treatment. Hypertrophic nonunions have abundant callus, indicating an adequate blood supply but a lack of sufficient mechanical stability for completion of fracture healing. Oligotrophic nonunions have little callus but still have an adequate blood supply; these nonunions are typically due to inadequate reduction with little or no contact between the fracture surfaces. Atrophic nonunions have no or little callus and have re-absorption of the bone; they are thought to be due to a deficient biologic process [7, 8].

The prevalence of nonunion and delayed union can be estimated from reports in the literature. In 22 series that included a total of 5517 fractures, the combined prevalence of nonunion was 2.5% and the combined prevalence of delayed union was 4.4%. In series of open tibial fractures, the prevalence of delayed union is higher, ranging from 4% to 52% in Gustilo-Anderson grade III fractures [7–9].

During the past two decades, numerous techniques have been developed to treat fracture nonunions. Invasive interventions include internal fixation with intramedullary nailing, and compression plates with or without the use of bone graft or bone graft substitutes [3, 6]. Noninvasive procedures include use of ultrasound and pulsed electromagnetic fields [10–12]. The percutaneous technique of autologous platelet gel (PG) application is a minimally invasive alternative [13]. This protocol exploits the osteogenic properties of platelet concentrate. The aim of this study was to evaluate the efficacy of autologous PG infiltration into delayed unions of the tibia which could evolve into atrophic nonunion.

Materials and methods

Between February 2002 and September 2005, we enrolled 15 patients (10 men and 5 women) with atrophic delayed union of tibial shaft fractures. The patients were aged between 21 and 67 years, with a median of 45 years. We considered cases of consolidation delays in unstable fractures of the tibial diaphysis, treated with blocked nail both proximally and distally, presenting a fibular fracture not completely restored. Fractures were distinguished in 3 different groups, considering if the area affected was the proximal third, the middle third or the distal third. We excluded from analysis patients with fractures in other districts, with exposed fractures, with mechanical stability issues, with altered values of erythrocyte sedimentation rate or C-reactive protein or with soft tissues problems in the limb considered.

The status of bone healing of the tibial fracture was evaluated through standard anterior and lateral radiographic views of the tibia. All delayed unions were considered atrophic because they showed little callus formation. The displacement of the bone fragments at the time of study entry was measured, on anteroposterior and lateral radiographs, and the maximum gap allowed between the fragments was 3 mm.

Platelet gel (PG) was prepared using quadruple bags and 380–450 mL autologous whole blood [10, 14]. The blood was fractionated into (a) red cell concentrate, immediately reinfused into the patient; (b) platelet concentrate (PC) in 14–15 mL plasma, in which the platelets were “hyperconcentrated”; and (c) platelet-poor plasma, used for the preparation of 10–15 mL cryoprecipitate (CRYO) by siphoning. The PC was subsequently combined with CRYO using a TSCD sterile connector (Terumo steriletubing welder, Terumo Italia, Rome, Italy) and the mixture thus obtained divided into 3 aliquots of 8–10 mL each; of these 3 aliquots, one was stired at 22° C under continuous agitation and used later. The following controls were carried out on the fresh final product (PC+CRYO mixture):

- Platelet count of the final product as a ratio of the patient’s baseline platelet count, obtained from a full blood count;
- Platelet count of the whole volume product;
- White cell count of the final product as a ratio of the patient’s baseline white cell count, obtained from a full blood count;
- Fibrinogen content of the whole volume product.

The planned therapeutic protocol was a cycle of three applications of autologous PG at intervals of 3–4 weeks. Each aliquot of PG was activated by adding 0.5 mL 10% calcium gluconate (Monico, Venezia Mestre, Italy) and 0.5 mL of a solution of batrox-

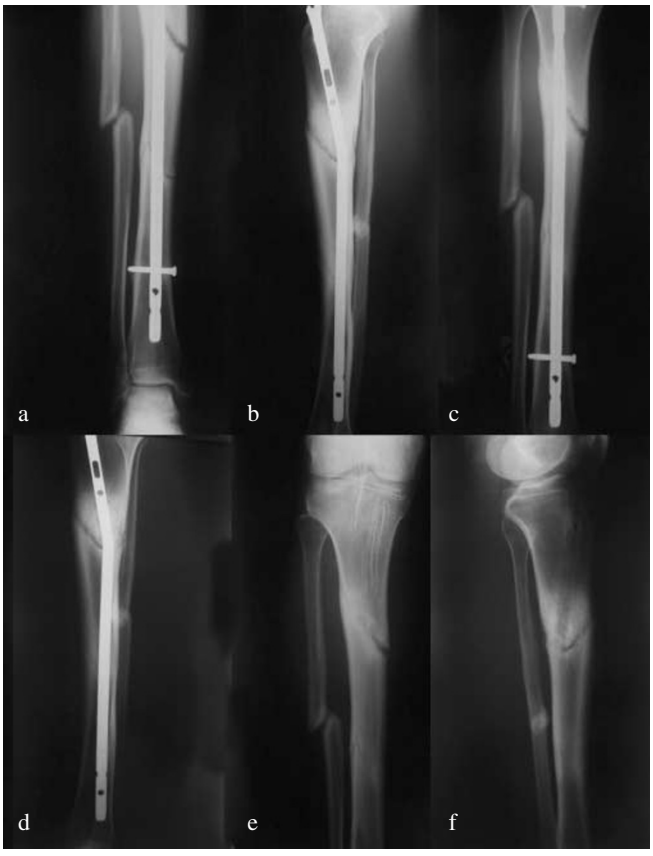

Fig. 1 a, **b** A 37 year-old man with a bifocal tibial fracture and proximal delayed union pre-treatment radiographs at 5 months from the intramedullary nailing (case 1). **c**, **d** Radiographs at of the end of treatment after 3 applications of platelet gel. **e**, **f** After removing the nail

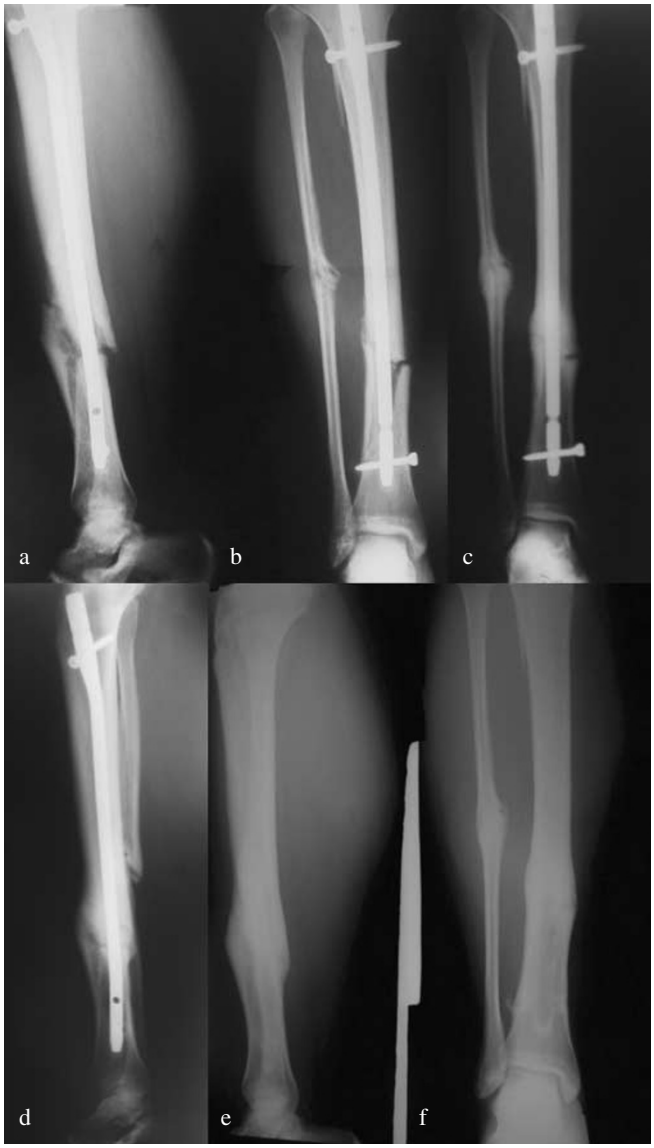

Fig. 2 a, **b** A 48 year-old man with a third distal shaft tibial delayed union at 5 months from the intramedullary nailing pre-treatment radiographs. **c**, **d** Radiographs at of the end of treatment after 3 applications of platelet gel. **e**, **f** After removing the nail

obin (Botropase, Ravizza Farmaceutici, Muggio, Italy). The product was activated and applied in the orthopaedic theatre in order to safeguard its sterility. In the operating theatre, after we positioned two 14 G needle-cannulae under radioscopic control at the border of the fracture, the PG was activated and immediately applied by infiltration. The gelification, which occurred within 2–7 minutes, did not hamper the infiltration: the second needle cannulae was, however, positioned in order to have a second injection route in case the first became obstructed by small lumps of platelet aggregate or cryoprecipitate, given the density of the product.

Radiological follow-up was planned for 2, 4 and 6 months after the first application. As the patients had atrophic delayed union and mobility at the fracture site, weight-bearing was not allowed during the first month following the injection to avoid mechanical disruption of the tissue-regeneration and bone-healing processes. After two months, if (and only if) callus was observed on radiographs, partial weight-bearing was allowed. There was a one-month transition period between the beginning of partial weight-bearing and that of full weight-bearing. The treatment was considered to be a success when there was definite radiographic evidence of fracture union (at least three of the four cortices viewed on the anteroposterior and lateral radiographs) and fulfillment of the clinical criteria of healing within four months after PG application. Clinical criteria for healing included full weight-bearing and no tenderness at the fracture site on palpation (Figs. 1, 2).

Results

We applied autogous platelet gel (PG) to 15 cases of atrophic delayed union of tibial shaft fractures. The results of quality control analysis of the 15 samples of fresh PG are shown in Table 1.

Of the 15 patients, 2 had proximal fractures, 8 had medial fractures, and 5 had distal fractures of the tibia (Table 2). Mean time between the fracture and the first application of PG was 151 days. The 3 planned PG applications were sufficient to induce healing in 8 cases, while 6 patients required 4 applications and 1 patient needed 5 applications for healing. There was complete consolidation of the focus of delayed union within 160 days in the 15 cases. In all cases, the synthesis aid was removed a mean of 12 months after the start of the treatment.

None of patients had complications during the application of autologous PG. There were no infections, haematomas, or chronic pain at the site of PG injection. All patients reported a clear improvement in pain symptoms, and thus limb function, already after the first infiltration.

The small number of cases in this study do not allow a correlation analysis to highlight any significant associations between response to treatment and age, sex or time

Table 1 Quality control on 15 preparations of autologous platelet gel

Parameter	Mean (SD)
Baseline platelet count (patient's blood count), x 10^3/µl	295 (91)
Platelet count of final product, x 10^3/µl	3218 (498)
Total platelet count of final product (x 10^9)	80.2 (21.0)
Baseline white cell count (patient's blood count), x 10^3/µl	7.37 (0.94)
White cell count of final product, x 10^3/µl	21.38 (17.30)
Fibrinogen continent of final product, mg	406 (204)

Table 2 Clinical characteristics of 15 patients with delayed union of a tibial shaft fracture treated with intramedullary nailing

Patient	Age, years	Fracture type	Time between fracture and PG injection, days	PG applications, *n*	Time for consolidation, days
1	56	Distal	133	4	120
2	35	Distal	161	4	120
3	58	Distal	156	4	120
4	31	Distal	136	4	120
5	67	Distal	151	5	160
6	56	Medial	155	3	90
7	37	Medial	147	3	90
8	44	Medial	141	3	90
9	47	Medial	144	3	90
10	41	Medial	158	3	100
11	23	Medial	169	3	100
12	61	Medial	166	3	90
13	21	Medial	142	3	60
14	67	Proximal	146	4	100
15	32	Proximal	160	4	120

to consolidation. However, a weak correlation was noted between the location of fracture and time of healing, because medial third delayed unions healed earlier (average, 89 days) than either proximal (average, 110 days) or distal (average, 128) delayed unions.

Discussion

Our study showed that percutaneous application of autologous PG is a safe treatment for uninfected atrophic delayed unions of the tibial diaphysis, as we encountered no local or systemic complications.

Historically, resection of the fibrous tissue at the nonunion site combined with mechanical stabilization has been described as being essential for the treatment of an atrophic nonunion [3]. In this series, the trocar was not used to remove the intervening callus or fibrous tissue. The fibrous tissue interposed between the bone ends ossified after the injection of autologous PG. It is difficult to explain the exact mechanism that allows the transformation of fibrous tissue into callus. Autologous PG was injected both in the nonunion gap and around the bones. It is not possible to know whether the injected autologous PG was able to convert the fibrous tissue into bone or if the interposed tissue was transformed into bone only after the bridging callus stopped micromotion at the nonunion site and allowed union of the gap.

One potential weakness of the present study is the absence of a cohort with a placebo treatment such as injection of saline solution. Percutaneous injection of autologous PG cannot be used when there is pre-existing angular deformity or shortening, both of which require direct access to the nonunion site. As the volume of callus obtained with this technique is limited, the fracture fragment gap size and displacement should be limited as well.

Platelets produce, store and release, if activated, numerous growth factors capable of stimulating the replication of cells of mesenchymal origin such as fibroblast, osteoblast and endothelial cells [15, 16]. Besides osteoprogenitor cells (fibroblast, osteoblast and chondrocytes), the regeneration of bone tissue requires a matrix conductive to bone formation, including osteogenic growth factors (e.g. platelet derived growth factor, PDGF; transforming growth factor (TGF) β; insulin-like growth factors (IGF) I and II) derived from platelets and morphogenically active proteins (bone morphogenetic proteins, BMPs). The BMPs are osteoinductive peptides derived from platelets and belong to the TGF-β group responsible for the processes of bone generation and regeneration [17–20]. In comparison with other currently available adjuncts, BMP may have a more substantial role to play in the treatment of delayed unions. Recombinant human BMP-7 (rhBMP-7) promoted union in a prospective, randomized controlled study of 122 patients with a total of 124 tibial nonunions [18]. All cases of nonunion were at least nine months old and had shown no progress toward healing for three months. Each patient had been treated with an intramedullary nail as well as with rhBMP-7 in a type-1 collagen carrier or with autogenous bone graft. At nine months postoperatively, 81% of the patients treated with rhBMP-7 and 85% of those treated

with autogenous bone graft were judged to have bone healing according clinical criteria. Thus, BMP plays an important rule in the treatment of tibial nonunion. Local factors, cytokines and growth factors are necessary to trigger and maintain tissue regeneration processes. Furthermore, growth factors released locally in bone tissue are incorporated into the mineral matrix and released slowly during the breakdown of the matrix by osteoclasts.

It has been hypothesised that there is an initial release of PDGF, TGF-β and IGF-I and -II at the sites of a bone lesion due to the effect of degranulation of platelets present in the lesion [19, 20]. PDGF stimulates mitosis of bone marrow stem cells and also has an angiogenic effect, inducing and enhancing the formation of new capillaries in the site of the lesion. At the same time, TGF-β causes proliferation of fibroblasts and pro-osteoblasts and differentiation of these latter into a more mature form (osteoblasts) which are stimulated to produce bone matrix, while fibroblasts deposit a collagen scaffold to support vessel growth. The local presence of platelets carried by the blood stream continuously provides the area of bone regeneration with necessary growth factors. Subsequently released IGF-I and -II act on osteoblasts of the endosteum, which starts to fill the trabeculae of cancellous bone [21].

We used repeated infiltrations of hyperconcentrated, activated autologous platelets in the form of a gel (PG) into the damaged site in order to enhance the role of platelet tissue repair mechanism. The application of PG in adjuvant treatment of bone tissue regeneration, incorporated appropriately into the overall management and multidisciplinary care of the patient, is being shown to be effective. On the basis of our experience, it is difficult to design protocols with certain prognostic evaluations because of a small number of available patients, and the limited cases reported in literature. However, the short healing times and the good results obtained lead us to consider this new method as one of most effective and promising treatments for delayed union. Furthermore, this is a simple and economic strategy for managing this unpredictable pathology.

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