Nonunions Treated With Autologous Preparation Rich in Growth Factors

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Objectives: To evaluate the clinical safety and efficacy of using a biologic technology known as preparation rich in growth factors (PRGF) for the treatment of nonhypertrophic nonunion.

Design: The design of the study was a retrospective case series.

Setting: The private practice was in 2 centers.

Patients: There were 15 patients with a total of 16 aseptic nonunions, 12 diaphyseal and 4 supracondylar, diagnosed as nonhypertrophic. The mean time since prior surgical treatment was 21 months (9–46 months).

Intervention: Supracondylar and diaphyseal nonunions followed surgical fixation with condylar plating or intramedullary nailing, whereas a composite biomaterial created by mixing PRGF with bone allograft was applied. The area was then covered with autologous fibrin membranes. Stable nonunions were treated with repeated percutaneous injections of PRGF; this minimally invasive procedure was also applied if delayed healing was suspected after surgical treatment.

Main Outcome Measurements: Radiographic union using radiographic views was taken in 2 planes. Clinical outcome evaluated pain, motion at the fracture site upon manual stress testing, and recovery of range of motion.

Results: All nonunions treated operatively healed after a single procedure, even though additional PRGF had to be injected in 2 patients. Two of 3 stable nonunions achieved healing only after repeated percutaneous PRGF injections. The mean time from surgery and/or PRGF application to union was 4.9 months (2–8 months). Complications associated with the described procedure were not observed.

Conclusion: This study, although uncontrolled, shows that PRGF technology is clinically safe and can enhance the healing of nonhypertrophic nonunions.

Key Words: bone graft, fracture fixation, growth factors, nonunion, platelets, percutaneous injection

(*J Orthop Trauma* 2008;0:000–000)

INTRODUCTION

Nonunion of fractures occurs when the normal biologic healing processes of bone cease, such that solid healing will not occur without further treatment. In these situations, a careful assessment of the mechanical and biologic factors contributing to the cause of nonunions can be used to direct treatment.[1–2] Several crucial elements should be considered, for example, whether the nonunion is septic or aseptic[3] and whether it is hypertrophic, with an intact vascular supply, or atrophic, with little callus and an avascular and nonviable nonunion site. The latter is thought to be associated with a deficient biologic process and may require the application of advanced biologic technologies.

The last few years have seen critical developments in platelet-rich therapies as biologic systems for growth factor release and tissue engineering. All these emerging technologies commonly use or release bioactive proteins at localized orthopaedic sites. The easy preparation of protocols, biosafety, and versatility of platelet-rich preparations and their reduced cost have encouraged their therapeutic use for the stimulation of tissue healing and bone regeneration. Preparation rich in growth factors (PRGF) is a standardized and well-characterized platelet-rich preparation that has shown its versatility and efficacy in several medical areas.[4–9] The bioactivity of PRGF is based on the progressive and balanced release of a pool of proteins and GFs, such as platelet-derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), and IGF-I, known to stimulate fracture healing.[10–12] In the present report, we describe the use of PRGF for nonunion treatment, namely, enhanced bone grafting that can be achieved by creating a composite biomaterial comprising PRGF and bone allograft[13]; in addition, fibrin membranes can be used to favor epithelialization, although liquid PRGF can be injected when additional delivery of GFs is required or less invasive procedures are indicated for stable nonunion. The present study retrospectively analyzes the efficacy and biosafety of

Accepted for publication September 30, 2008.

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Supported by the Basque and Spanish Governments.

Marketing approval: Preparation rich in growth factors are licensed by the European Regulatory Agency as a class III medical device, Directive 93/42/EEC (EC Certificate G1 05 03 43180 006). Preparation rich in growth factors are indicated on the area of oral surgery and treatment of skin ulcers and musculoskeletal injuries.

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PRGF technology in 15 patients treated for nonhypertrophic nonunions of long bones.

PATIENTS AND METHODS

The study group was identified from a larger group of 32 consecutive patients with nonunion treated at 2 private medical centers between November 2003 and 2005. Of these 32 patients, 15 patients with 16 nonhypertrophic nonunions of long bones, 12 diaphyses (4 humerus, 4 femurs, and 4 tibias), and 4 supracondyles were treated using this procedure. Nine patients with hypertrophic nonunions, 6 nonunion fractures in short bones, and 2 patients under treatment with the anticoagulant Sintrom were excluded.

Nonunions were classified according to the Weber–Cech classification[14] by 2 experienced orthopaedists involved in the care of the patients. A nonhypertrophic nonunion was considered if there was no radiographic bone healing or periosteal bone formation and if the bone ends were atrophic or oligotrophic in appearance (with little or no callus formation) or if a gap between the fracture ends and tapering of the bone ends was present. We did not participate in the original management of any of these patients but rather received them as referrals from other institutions.

The protocol was approved and conformed to the national and international (ICH rules) policies on clinical studies by our institutional review boards. All patients gave written informed consent. Three experienced senior surgeons performed all surgeries and/or PRGF injections, and 2 other independent observers assessed the clinical results and radiographic images. Preoperative standard radiographs of anteroposterior and lateral projections were obtained and a computerized tomography scan was taken in those cases demonstrating minimal doubt about cortical healing (cases 3, 4, 5, and the femur of case 7).

Stability was achieved by intramedullary nailing or condylar plate fixation, and bone grafting combined with PRGF (prepared as described below) was applied in all surgeries excluding 2 cases (8 and 9), which were treated percutaneously with only PRGF as further explained.

PRGF Technology

For the preparation of PRGF from the patients, 65 mL of peripheral venous blood was withdrawn into 9-mL tubes containing 3.8% (wt/vol) sodium citrate. PRGF was prepared by centrifugation at 640*g* for 8 minutes at room temperature (BTI System, Vitoria, Spain). From this plasma, different biomaterials are obtained depending on the coagulation and activation degree of the samples as described below. The upper plasma fraction was drawn off and deposited in a collection tube; it was used to prepare the fibrin membrane. The 2-mL plasma fraction located just above the sedimented red cells, but not including the buffy coat, was collected in another tube; it may be combined with bone graft and/or injected in liquid form. PRGF is a versatile plasma that can be tailored according to the specific application procedure: surgery (modality 1) or injection without surgery (modality 2).

Modality 1: PRGF + Surgical Treatment

PRGF Preparation

PRGF Combined With Bone Graft.

Once the PRGF was activated with 10% (wt/vol) calcium chloride, it was mixed with the morselized bone allograft in a specific dish or bowl depending on the shape and size of the graft needed. PRGF was allowed to clot ex vivo until the composite graft became compacted and easy to handle. Then, it was precisely shaped to permit good fitting in the nonunion bed site (Fig. 1A). Homologous frozen cancellous allograft was used in all but 2 of the cases for which an autograft was used (ie, the graft was obtained from a low-speed reaming procedure[13] in case 4 and from the iliac crest in case 5, Table 1).

Fibrin Membrane.

To prepare the autologous fibrin membrane, 8–12 mL of plasma located at the top of the tubes was transferred to a glass bowl. After adding calcium chloride, the mixture was

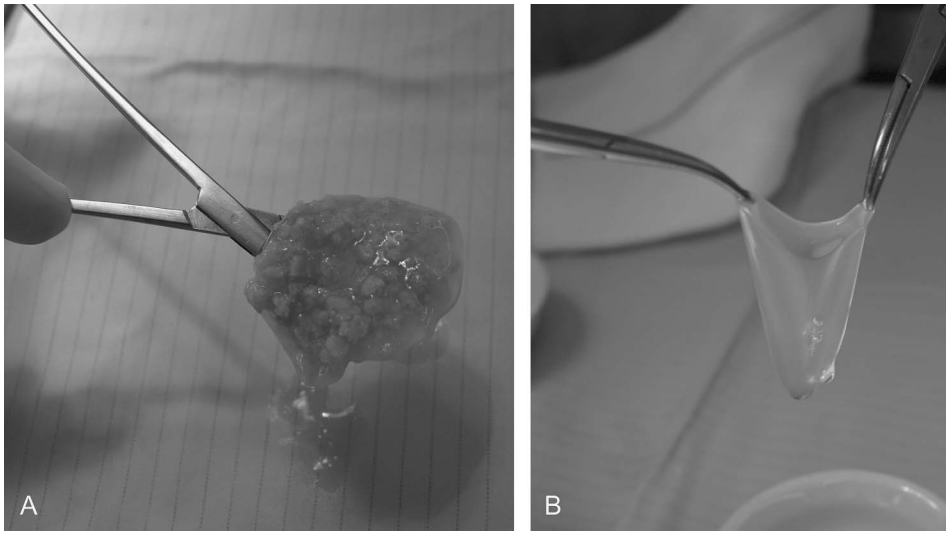

FIGURE 1. A, Composite biomaterial made of allogeneic morselized cancellous bone combined with PRGF; (B) autologous PRGF fibrin membrane used to cover the reconstructed area.

TABLE 1. Summary of Demographic Data and Clinical Status Before Nonunion Treatment

Case (Sex)	Age (yrs)	Cause	Bone (Fracture Type)	Side Injured	Prior Surgeries	Previous Fixation*	Time Until Nonunion Treatment (mo)
1 (F)	63	Traffic accident	Fm (C)	L	1	Plate	18
2 (M)	52	Traffic accident	Fm (O)	R	2	Plate	30
3 (M)	45	Traffic accident	H (O)	R	1	IM nail	24
4 (M)	65	Low-energy fall	H (C)	R	1	FlexRod	15
5 (M)	29	Traffic accident	T (O)	L	2	Ext Fix	14
6 (M)	29	Traffic accident	T (O)	R	2	IM nail	20
7 (M)	21	Traffic accident	T (O)	R	1	Ext Fix	9
			Fm (C)	R	3	IM nail	15
8 (M)	21	Traffic accident	T (C)	R	1	Ext Fix	30
9 (F)	28	Traffic accident	Fm (O)	L	2	IM nail	16
10 (F)	73	Traffic accident	H (C)	R	2	IM nail	23
11 (F)	46	Traffic accident	Fm (O)	R	5	FlexRod	46
12 (F)	65	Low-energy fall	Fm (O)	R	2	IM nail	18
13 (F)	62	Low-energy fall	Fm (O)	L	2	Plate	15
14 (F)	69	Low-energy fall	Fm (O)	R	2	IM nail	26
15 (M)	27	Low-energy fall	H (O)	R	5	Plate	15

AU16 C, close; Ext Fix, external fixation; F, female; Fm, femur; H, humerous; IM, intramedullar; M, male; O, open; T, tibia.
*Type of fixation before treatment at our department.

incubated for 30–40 minutes, allowing for the formation of a biocompatible fibrin membrane. Hemostasis could be obtained by placing fibrin membranes on an osseous surface. These membranes helped to promote full epithelialization of soft tissues (Fig. 1B).

Surgical Procedure

In every patient, the less invasive approach was planned and care was taken to manage the soft tissues and periosteum near the nonunion site. For instance, in those sites previously treated by either plate or external fixation (n = 9), the implants were removed by complete excision of the neighboring tissue. In cases (n = 7) in which previous nailing had failed, a minimal incision of around 4 cm was made, followed by careful resection of fibrous tissue and decortication or shingling of the bone at the nonunion site. For patient 4 (Table 1), who had poor-quality skin, an endoscopic approach for both fibrous tissue resection and PRGF/graft application was used. In all diaphyseal nonunions, mechanical fixation before the PRGF/graft application was achieved using an intramedullary nail (T2; Stryker TraumaGmbH, Schönkirchen, Germany) inserted with reaming and locking it proximally and/or distally to the fracture, depending on the specific type and location. Alternatively, operative management of supracondylar femoral nonunion (cases 11, 12, 13, and 14) involved femoral plate fixation using a dynamic condylar screw (DCS) (Synthes GMbH, Oberdorf, Switzerland). When treated sites did not heal properly (cases 1 and 2), PRGF was injected percutaneously as explained below.

Modality 2: PRGF Injection Without Surgery

Activated Liquid PRGF.

For the closed treatment option, activated liquid PRGF was percutaneously injected without exposing the fracture site. In this procedure, the goal was to control the degree of platelet activation. Therefore, PRGFs were injected shortly after addition of $CaCl_2$. The volume injected is usually in the range of 6–8 mL, but the volume tolerated at different sites of AU5 injection may be slightly variable. The injected biomaterial did not wash away because a platelet-rich fibrin develops within the nonunion site, releasing growth factors into the fracture area.

Three patients (8, 9, and 10) were treated exclusively with percutaneous injections of PRGF under mild anesthesia. Imager intensifier was used to precisely locate the area of nonunion, and to stimulate the nonunion, several small perforations were made by gently inserting a trocar down to bone. Subsequently, 6–8 mL of activated liquid PRGF was injected into the fracture site and around the bone ends. This treatment was repeated every other week, for 6 weeks (3 injections).

In surgically treated patients who were not healing properly, several small perforations were made in the initial callus using a trocar and 6–8 mL of activated PRGF was injected. The procedure was carried out on an outpatient basis. In this process, after premedication with 1 mg of medazolam, AU6 anesthesia was induced with intravenous propofol (2 mg/kg) along with ketamine (10–15 mg) to provide analgesia.

Clinical Outcome

After nonunion treatment, all patients were followed up prospectively, according to protocol, that is, through appointments on the second week and then at 4- to 6-week intervals, until healing was confirmed. Subsequently, patients were followed up at 12 and 18 months and yearly thereafter. At each follow-up, standard radiographic views were taken in 2 planes. Healing was defined radiologically by the presence of a bridging callus, even if the fracture line remained visible, or by bone trabeculae crossing the original nonunion. Clinical

diagnosis of union was determined by the absence of pain and absence of motion at the fracture site upon manual stress testing in the sagittal and coronal planes and by functional recovery of range of motion of the involved extremity. If suboptimal outcomes were suspected by weeks 10–16 after treatment, a computerized tomography scan was obtained to consider either postsurgical PRGF infiltrations (as in cases 1 and 2) or surgery (as in case 10). In patients treated with PRGF injections only (cases 8 and 9), after both radiologic and clinical criteria for healing were met, a computerized tomography scan was obtained to further confirm healing.

The mean time from prior surgical procedure to PRGF/graft operative management or PRGF closed treatment was 21 months (range 9–46 months). All patients were followed for a minimum of 12 months (mean 15, 3 months, range 12–48 months).

RESULTS

Demographic data including age, sex, location, type of fracture, mechanism of injury, and previous treatments are summarized in Table 1. All patients had pain and felt severe discomfort; moreover, 3 patients (cases 2, 3, and 4) described a feeling of instability at the nonunion site. All patients had a confirmed nonhypertrophic nonunion on radiographs. Table 2 shows patients with risk factors for impaired healing. The union rate after surgical treatment was 84.6%. Three patients with suboptimal healing were salvaged by injecting additional PRGF in the postoperative period. Figure 2 shows a patient flowchart demonstrating an overview of the applied procedures.

The mean time from surgical fixation and PRGF/graft or closed PRGF application to union was 4.9 months, range 2–8 months (Table 3). The longest time to union was 8 months in 1 patient with a complex femoral distal shaft fracture (case 2).

Of the 3 patients requiring additional postoperative injections, 2 (cases 1 and 2) underwent percutaneous PRGF treatment 10 and 15 weeks, respectively, after the index procedure. Case 1 was a woman with poor bone quality who required extended tissue resection and decortication during surgery because of severe fibrosis and massive failure of the initial hydroxyapatite graft; in this case, 1 postsurgical PRGF injection was found to be sufficient. Afterward, she was asymptomatic and achieved radiographic healing by the sixth month. In case 2, a middle-aged man sustaining a diaphyseal-metaphyseal open distal femur fracture with poor fulfillment of the treatment protocols and weight-bearing restrictions, 3 percutaneous PRGF injections every other week were considered when slow progress of fracture healing was observed by week 15. The patient had systemic risk factors including diabetes mellitus, cigarette smoking, alcohol abuse, and cocaine addiction that may have affected the metabolic pathways related to bone formation. Although 2 PRGF applications were performed biweekly, a tibial nondisplaced fracture subsequent to a fall had forced a 4-week postponement of the third planned PRGF infiltration. The patient had 2 more accidents until the fracture was healed with no adverse effects by the eighth month.

It is noteworthy that in 2 (8 and 9) of 3 patients percutaneously treated with PRGF (nonsurgical treatment), the union was achieved in 6 and 3 months, respectively, whereas it failed in another case (10) in which the outcome was suboptimal by week 14 and the patient had to be treated surgically.

In all patients, there was a good correlation between the radiologic findings, in terms of radiologic signs of healing, and clinical ones. Two representative examples (being cases 4 and 6, Table 1) are illustrated in Figures 2 and 3, respectively.

Major complications such as osteomyelitis, infection, neurovascular injury, or thromboembolic conditions were not reported, and there were no cases of implant loosening or

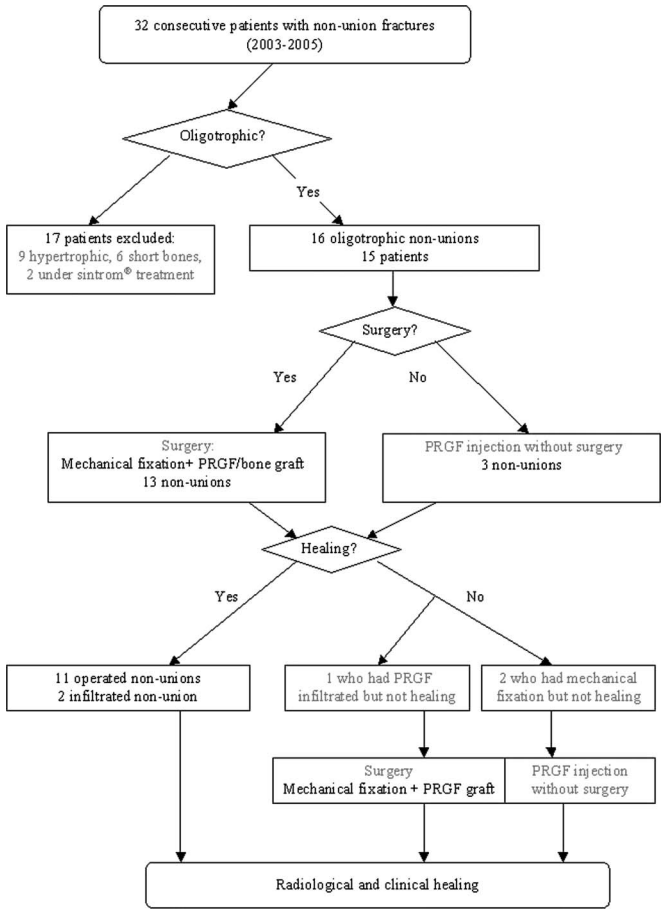

FIGURE 2. Patient flowchart showing an overview of the applied procedures.

TABLE 2. Risk Factors Relevant to Bone Healing in This Group of Patients

Risk Factors		Case Number
Systemic	Type 2 diabetes mellitus	2
	Smoking	2, 3, 6, 9
	Drugs	2
Local	Presence of necrotic tissue	5, 13, 14
	Neurologic injury	5
	Soft-tissue injury	2, 7
	Comminuted	5, 11, 13, 14
	Displaced fracture	2, 4

TABLE 3. Summary of Surgical Fixation and Biologic Treatment and Time to Nonunion Healing

Case Number	Bone	Mechanical Fixation	PRGF/Graft	No. PRGF Injections	Time to Healing (mo)*
1	Fm	IM nail	Allograft	3 (postoperative)	6
2	Fm	IM nail	Allograft	3 (postoperative)	8
3	H	IM nail	Allograft	—	6
4	H	IM nail	Autologous chips	—	5
5	T	IM nail	Iliac crest	—	3
6	T	IM nail	Allograft	—	6
7	T	IM nail	Allograft	—	5
	Fm	IM nail	Allograft	—	6
8	T	—	—	3	6
9	Fm	—	—	2	3
10	H	IM nail	Allograft	3 (preoperative)	6
11	Fm	DCS plating	Allograft	—	5
12	Fm	DCS plating	Allograft	—	3
13	Fm	DCS plating	Allograft	—	2
14	Fm	DCS plating	Allograft	—	2
15	H	IM nail	Allograft	—	6

Patients 8 and 9 were treated nonoperatively. Type of graft (allograft: cancellous bone, autologous: iliac crest, and chips obtained from a low-speed reaming procedure), all grafts were combined with PRGF.

AU17 Fm, femur; H, humerous; IM, intramedullar; T, tibia.

*Clinical and radiologic confirmation of fracture healing after IM nailing and PRGF/graft application.

failure up to the completion of this review. Minor complications such as early discomfort at the entry point of the nails were reported by 2 patients; one of them (case 4) asked for removal of the humeral nail after bone union, but clinical resolution was achieved by arthroscopic subacromial decompression. In the endoscopically treated patient (case 6), we encountered partial-thickness skin loss around the portals but these healed with local wound care.

DISCUSSION

Our preliminary results indicate that PRGF is a clinically safe biologic technology that demonstrates a high rate of success for the treatment of nonunions. In fact, all patients showed clinical and radiographic signs of complete fracture healing at 2–8 months.

Platelet-rich preparations, also called platelet-rich plasma, have gained much attention in the scientific community as they allow for the release of a myriad of growth factors and cytokines after activation into the local environment.[15,16] However, the lack of suitable standardization and a definition for these products has provoked the appearance of a wide range of biologic preparations that differ from a qualitative and quantitative point of view. In addition, the protocols of administration in the patients differ extensively, leading to controversial therapeutic effects. PRGF is characterized by a moderately elevated platelet concentration (2- to 3-fold peripheral blood count), showing consistency in the relative concentrations of PDGF and TGF-β1 and a good correlation with the number of platelets present in the matrices.[17] In contrast to most other platelet concentrates, PRGF does not contain leukocytes,[5,9,18] thus improving homogeneity, reducing donor-to-donor variability, and reducing neutrophil secretions such as matrix metalloproteinases-8 and -9 and free radicals that destroy all surrounding cells whether they are injured or healthy. After activation with only calcium chloride (avoiding the use of exogenous thrombin) and combination with morselized bone, a 3-dimensional fibrin scaffold develops that agglutinates the graft particles, resulting in a handy product that is easy to manipulate during surgery and is adaptable to the injured site.

Another advantage of PRGF technology is its versatility,[6] that is, it further permits local delivery of growth factors nonoperatively by infiltrating the fracture site with activated liquid plasma.[19] Addition of calcium chloride promotes the gradual formation of native thrombin, mimicking the physiologic clotting process and enabling a more sustained release of growth factors.[20] Alternatively, exogenous thrombin mixed with calcium chloride can be used to induce clotting. However, the latter procedure is less versatile, and the biomaterial cannot be injected percutaneously because it clots in 3–5 seconds. One may theorize about the therapeutic potential of PRGF when applied to other clinical conditions such as fresh fracture or corrective osteotomies, for the reason that it involves direct stimulation of healing through biologic activation of callus cells. Furthermore, as shown in our report, fracture nonunions with good alignment and good bony apposition may be treated with a less invasive procedure involving repeated percutaneous injections of PRGF.

It is difficult to determine how many injections are sufficient. In fact, this minimally invasive option produced good results after 2–3 injections in 2 cases, whereas it failed in another patient (10) after 3 injections. PRGF injections can be critical when impaired bone healing is attributed to a deficient blood supply, as is the case in atrophic nonunions. In effect, an important feature of PRGF is its angiogenic capacity that is AU8 controlled by an ambivalent relationship between proangiogenic (TGF-β1, vascular endothelial growth factor, HGF,

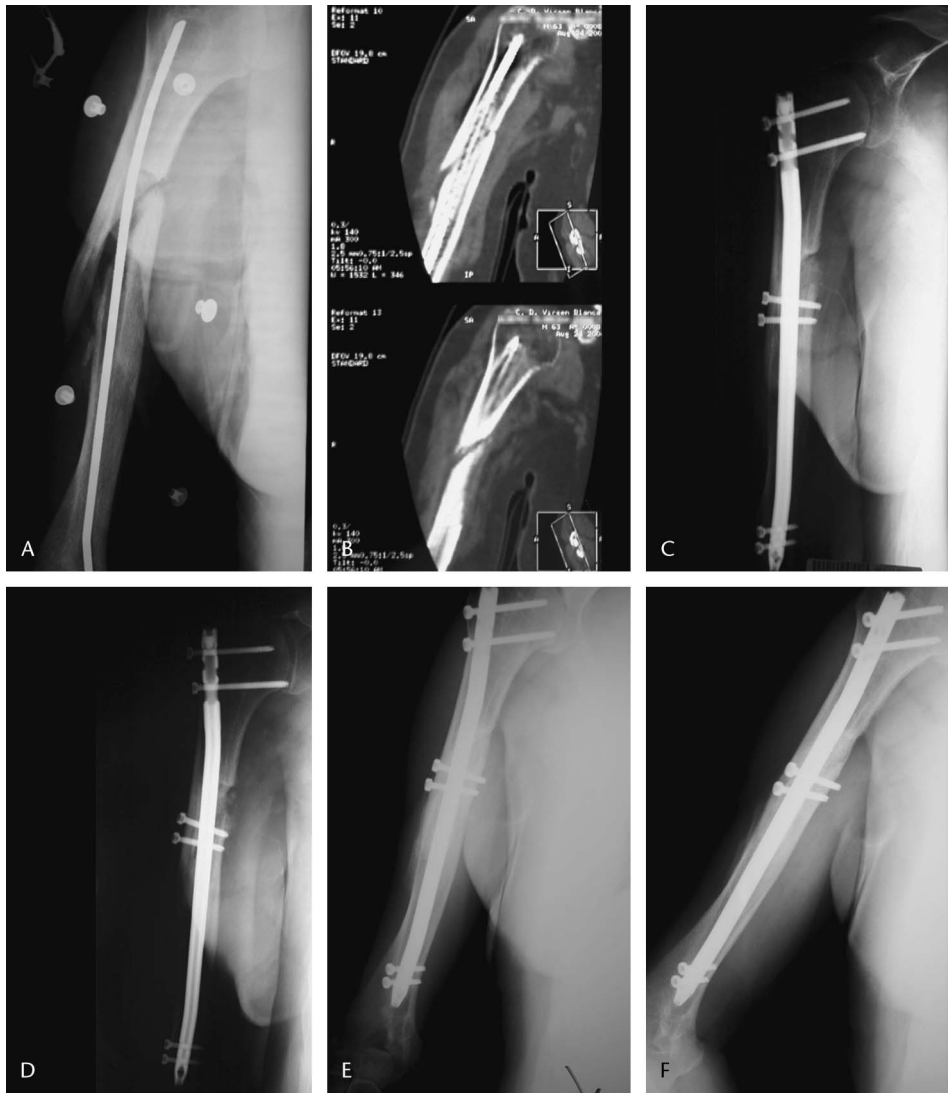

FIGURE 3. A–F, A 65-year-old man fell while walking and sustained a fracture at the humeral shaft with a long third fragment (reported as case 4 in Table 1). A, Preoperative radiograph and (B) computerized tomography of the patient's right humerus taken 9 months after unsuccessful reconstructive surgery. C, The images show an oligotrophic nonunion attributable to improper alignment; and (D) radiograph obtained 1 month after PRGF/allograft application shows mechanical stability achieved by nailing with 2 extra screws to fix the third fragment; (E) and (F) 5-month postoperative radiographs show fracture healing and PRGF/allograft incorporation.

angiopoietin-1, and CD-40L) and antiangiogenic factors (thrombospondin-1, beta-thromboglobulin, PF4, and endostatins).[15,16]

We suggest at least 3 PRGF injections in nonoperatively treated patients because PRGF degraded too quickly compared with the time required for bone regeneration. Although the amount/timing of injections are still being investigated, the present injection schedule was successful in contrast with recent published work that failed to improve long-bone nonunions after isolated percutaneous thawed platelet gel supplementation.[21]

On the other hand, when PRGF is combined with graft particles in surgically treated patients, the latter might act as a release carrier for growth factors from PRGF, favoring a more sustained release of proteins and hence continuous cellular activation. Even so, in the presence of risk factors, when it is presumed that the union is not going to occur in the expected time frame because of adverse local and/or systemic conditions, additional GF delivery to provide cell reactivation may be performed.

Studies have confirmed that local application of platelet-released proteins is especially important in pathologic conditions in which fracture healing is compromised due to an inadequate biologic environment that is marked by reduced levels of factors such as TGF-b1, IGF-I, and PDGF.[22] This concept has been shown in diabetic rats in which percutaneous injection of platelet-rich plasma normalized diabetic fracture callus.[23] Moreover, recent studies have reported reduced levels of systemic TGF-β1 in patients with nonunions, possibly reflecting local mechanisms during the pathophysiologic process.[12] In these complex situations, in situ application of autologous plasma ensures an initial release of factors such as PDGF, IGF-I, and TGF-β1 that is relevant for chemotaxis and osteoblast activity.[14–24] Such factors have demonstrated great potential when applied in combination with each other[11] or with other proteins (vascular endothelial growth factor, HGF, CTGF, and endothelial growth factor) also present in platelet-rich preparations.[25] Furthermore, preclinical and clinical studies with PRGF have shown that it can accelerate tissue repair in soft tissues by mechanisms that involve further

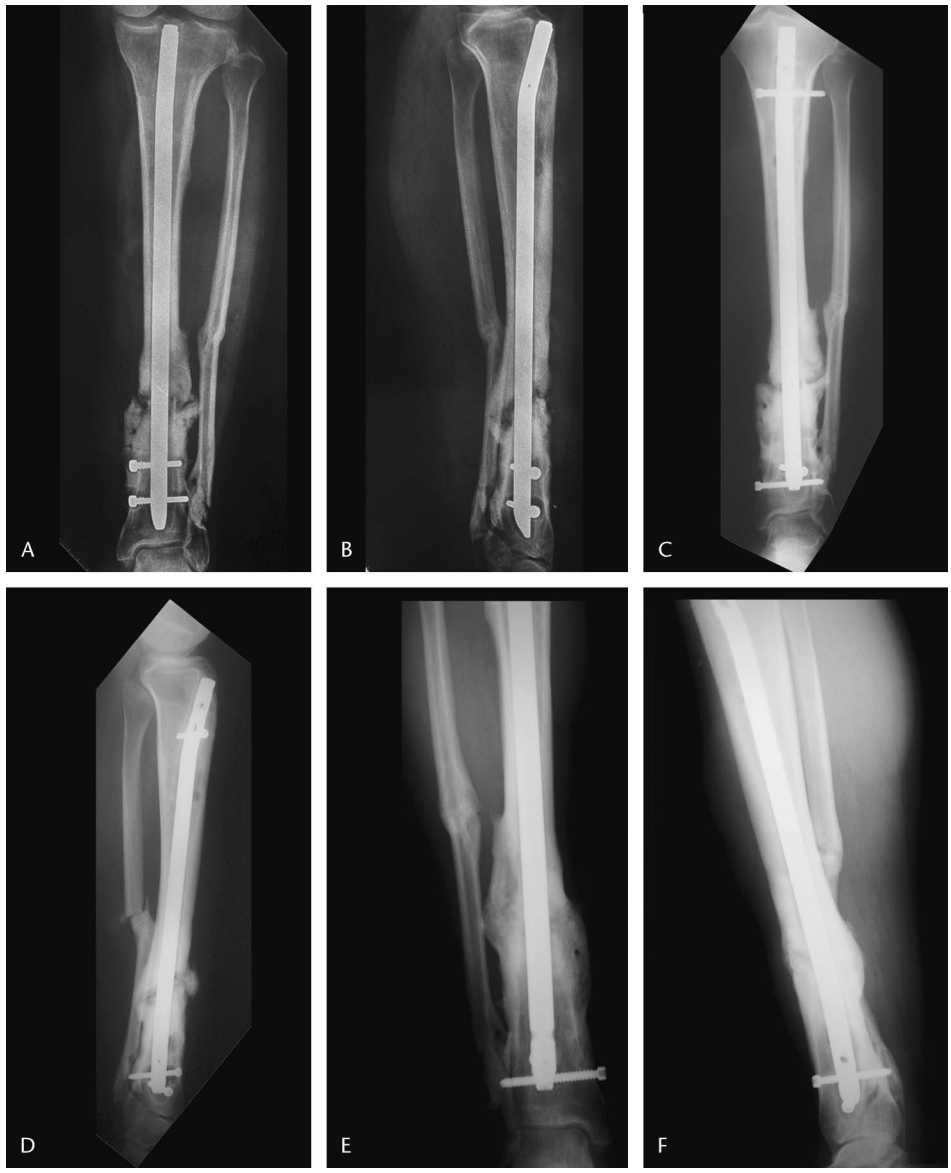

FIGURE 4. A–F, A 29-year-old man had a motorbike accident and sustained an open fracture at the distal diaphyseal third of the left tibia (reported as case 6 in Table 1). A, Anteroposterior and (B) lateral radiographs of the patient's tibia 12 months after the first reconstructive surgery. C, Anteroposterior and (D) lateral images showing the nonunion site 8 weeks after surgical reconstruction with PRGF/allograft. E, Anteroposterior and (F) lateral radiographs 6 months postsurgery; radiographs show bone consolidation and allograft incorporation in addition to good alignment and correct hardware positioning.

synthesis of signaling proteins that participate in cell mitosis and angiogenesis.[26,27]

Despite these encouraging results, much work is needed to determine which and why platelet-rich products are effective in some situations,[28–32] but ineffective in others.[33–35] Thus, the product characteristics and procedure of application,[36,37] in conjunction with the precise nature of the injury and specific patient comorbidities and the ideal time for autologous plasma injection and exact time when the potential effects are evaluated, may be of paramount importance to address this issue.

Without question, mechanics in coordination with biologics is the primary consideration in the management of fracture nonunions.[38,39] Insertion of an intramedullary nail with reaming and DCS plating were the treatments of choice; the former allowed for insertion of a tighter fitting nail across the isthmus of the long bone, leading to greater stability. We have also used a dynamically locked, rather than unlocked, intramedullary nail to diminish torsional forces around the fracture. In addition, DCS plating in condylar nonunions can lead to solid fixation where standard plate constructs might fail. Intramedullary nail insertion for long-bone nonunions, or DCS plating in the metaphysis of the femur, coupled with grafting using a composite made of allograft and PRGF may be a useful procedure in the management of nonhypertrophic nonunions, as shown in this case series study.

Although all these patients were treated consecutively and outcomes and objectives were independently assessed, the validity of the study is limited by the lack of a comparison group; hence, additional researches using randomized treatment groups are required to further confirm the effectiveness of PR therapies in nonunion treatment. Moreover, from a biologic viewpoint, much work remains to be performed to determine the identity of the essential factors required to

accelerate healing; this knowledge will aid in developing more efficient platelet-rich preparations tailored to the specific requirements of the different tissues.

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