

Mini-invasive treatment for delayed or nonunion: the use of percutaneous autologous bone marrow injection

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Abstract— Delayed union or nonunion of bone fracture is becoming less frequent, but still remains a challenging clinical problem. Autologous cancellous bone grafting that is the gold standard method, often involves donor site morbidities and complications. Once these fractures have been mechanically stabilized, other local factors should be investigated to promote delayed healing. The purpose of this study was to evaluate the initial outcome of the percutaneous injection of autologous bone marrow after concentration for the treatment of delayed or nonunion. Our subjects included 10 patients (3 female, 7 male) with an average age of 28 years. All fractures were mechanically stabilized after accident. Delayed or nonunion affected the femur in 2 patients, the tibia in 5 patients, the humerus in 2 patients, and the ulna in 1 patient. Bone marrow aspirates were obtained from both the posterior superior iliac crest. Bone marrow aspiration concentrate was produced via density gradient centrifugation. Trocars were inserted in the delayed or nonunion gap under fluoroscopic guidance. The bone marrow aspiration concentrate was injected slowly. All of 10 delayed or nonunion healed after treatment with percutaneous injection of autologous bone marrow. The mean time for new bone formation was 3.3 months, for clinical union was 5.2 months, and for radiological union was 11.8 months. The result of current study is encouraging in the initial outcome and percutaneous bone marrow implantation could be an effective and safe treatment for delayed or nonunion.

Keywords: Delayed union, nonunion, mesenchymal stem cells, bone marrow aspiration concentrate, and percutaneous autologous bone marrow injection

INTRODUCTION

Despite recent advances in the biomechanical stabilization of fracture and understanding in the biological processes of healing, delayed union or nonunion is becoming less frequent, but still remains a challenging clinical problem. Autologous cancellous bone grafting is still the gold standard biological method for bone healing by introduction of vital local components: osteoconduction, osteoinduction, and osteogenic cells (Pountos, 2010). This bone graft often involves remarkable donor site morbidities and complications, as well as additional procedures.

The development of alternative techniques of biological stimulation, which must also provide these osteogenic components, is required. Once the stabilization of fracture has been mechanically met, other local factors should be investigated to promote delayed healing (Pountos, 2010). More recently, numerous bone graft substitutes have been presented with varying degrees of successful outcome (Zimmermann, 2010). Connolly and Shindell first demonstrated that nonunion could be treated successfully in the clinic with the percutaneous injection of bone marrow (Connolly,

1989, 1991). However, bone marrow after aspiration was directly injected to fracture site. This led to low rate of the implanted bone marrow. The purpose of this study was to evaluate the initial outcome of the percutaneous injection of autologous bone marrow after concentration for the treatment of delayed union or nonunion fractures.

MATERIAL AND METHODS

The prospective study was performed in patients who were treated at our Center from January 2013 to December 2014 with delayed union or nonunion fractures following fracture stabilization. The protocol for this clinical study was approved by the Committee of Medical Training and Research Center. Our subjects included 10 patients (3 female, 7 male) from 18 to 48 year old with an average age of 28 years. Delayed union or nonunion affected the femur in 2 patients, the tibia in 5 patients, the humerus in 2 patients, and the ulna in 1 patient. All fractures that were presented with Müller AO fracture classification were stabilized. Two closed fractures at the femur and tibia had been stabilized by inter-locking intramedullary nail. Three cases of open fractures at the tibia, which had a very bad scar, were stabilized by an external device. All the remaining fractures were osteosynthesized by plates. Patient demographic, medical history, initial fracture treatment, and operative and follow-up data were recorded.

A diagnosis of delayed union or nonunion is based mainly on the clinical and radiographic examination. Clinical evidence of delayed union was determined by documented pain, motion at the fracture site, and tenderness on stress at the fracture site. An injury is deemed "delayed union" when an adequate period of time has elapsed without achieving bone union, with a time period of six months or less since the initial injury. "Nonunion" was determined by lack of radiographic evidence of bridging osteophytes at six months or a fracture that has not displayed progression of healing over a 3-month period (Hernigou, 2005b; Tressler, 2011). Clinical assessment and pre-operative laboratories tests such as white blood cell count, erythrocyte sedimentation rate, and C-active protein were performed to rule out infection. Exclusion criteria included fractures with delayed or nonunion due to a mechanical cause related to the bone fixation necessitating revision of the stabilization, and

the presence of a gap at the fracture site more than 10 mm wide which needed to be filled with a structural graft (Akram, 2014; Kassem, 2013).

No patients were included in analysis if they were actively being treated for an infection at the fracture site or ongoing treatment with immunosuppressant drugs: glucocorticosteroids, chemotherapy or colchicines at the time of nonunion revision (Hernigou, 2013; Tressler, 2011). Patients were also excluded if they were pregnant and lactating, or had autoimmune deficiency syndrome, hepatitis, or a medical history of alcohol or drug abuse (Jager, 2011).

Isolation and concentration of bone marrow

The procedure of bone marrow aspiration was performed under general anaesthesia. Patients were placed in a prone position on an operating table. Bone marrow aspirates (350 ml) were aspirated from both posterior superior iliac crests of the patients by the Jamshidi vacuum. The samples were collected by qualified hematologists. Mesenchymal stem cells (MSCs) processing techniques were established as previously described (Giannotti et al., 2013). Bone marrow aspiration concentrate (BMAC) was made via a density gradient centrifugation using the Sorvall™ centrifuge (Thermo Scientific, MA, USA) at 3,670 rpm for 7 min. Finally, a total volume of 10 ml BMAC was poured into a syringe for injection (Jager et al., 2011).

MSCs reimplantation

MSCs reimplantation was performed using a technique described by Hernigou et al. (Hernigou, 2006). Under general anaesthesia, the patient was placed on the operating table in a supine position. The area of the nonunion site was prepared and draped in a sterile fashion. The tip of the trocars (15 G Barker-Bier Luer Lock, Unimed SA, Lausanne, Switzerland) was navigated into the delayed or nonunion gap and around the bone ends at several directions guided by the images of C-arm fluoroscopy (Philips Medical Systems NL B.V., Best, The Netherlands) (Fig. 1). The bone marrow aspiration concentrate (BMAC) was injected slowly at a speed of 20 ml/minute in a controlled fashion. After injection, the trocars, with the stylet in place, was withdrawn slowly to blood clot gradually bit of trocar paths (Hernigou, 2006). The patient was asked to keep in the same position for the first hour to allow for cell attachment. There was one case of the humeral fracture stabilizing by plate and screws; one screw that had transversed the fracture line needed

removal before injection. Two cases with an intramedullary nail at the femoral and tibial fracture were dynamized by removal of the distal locking screw. In two cases of tibial fracture, external device was removed and the fracture was stabilized by plaster cast (Centeno C.J., 2011).

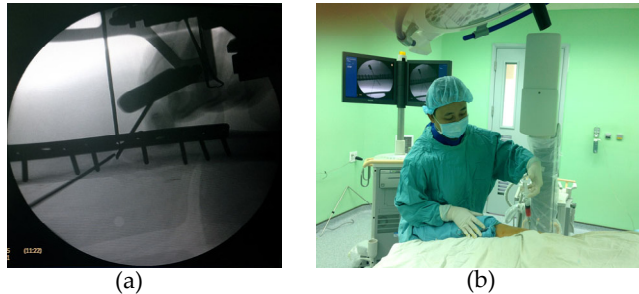


Figure 1. (a) The trocars were positioned into the bone defect gap under fluoroscopic guidance; (b) Percutaneous injection of autologous bone marrow for the treatment of delayed union or nonunion fractures.

Assessment of fracture healing

These patients were monitored using the same protocol during the postoperative period. Patients were followed up at 1 month, 3 months, 6 months, 9 months, and 12 months, and continuously followed up until full bone union was achieved, as determined by clinical examination and radiographs. Weight bearing was not allowed during the first month after bone marrow transplantation. If callus was observed radiographically after one month, partial weight bearing was allowed. At the third month, if the patient had no more pain and there was cortical callus or disappearance of the fracture lines on radiographs, full weight-bearing was allowed. And if a plaster cast was used it would be removed. The physical examination assessed pain, sensation of stability, and ability to walk with or without crutches. Anteroposterior and lateral x-rays were performed at follow-ups. Assessment of bone union and remodelling was based on the modified Lane and Sandhu radiological scoring system. Three experts blindly assessed the radiological scores, which were the sum of the scores of bone formation and remodelling. The score for bone formation was determined as 0 (no bone formation), 1 (<25% bone formation), 2 (25–50% bone formation), 3 (50–75% bone formation), and 4 (>75% bone formation). The score of union was 0 (full fracture line), 2 (partial fracture line), or 4 (absent fracture line). The remodelling score was 0 (no evidence of remodelling), 2 (remodelling of the intrame-

dullary channel), and 4 (full remodelling of cortex). The maximum point could be achieved was 12.

Bone Marrow Analysis

The bone marrow cells that achieved after a density gradient centrifugation were analysed. In brief, the total mononuclear cells from bone marrow samples were counted with the use of a SysmexXS 800i Haematology Analyser (Sysmex Europe GmbH, Norderstedt, Germany). Cells then were displayed by immunofluorescence staining with antibodies against CD 34 (Becton, Dickinson and Company, New Jersey, United States). CD34⁺ cells were counted and expressed as % CD34⁺ cells and CD34⁺ cells/ml. For cell viability evaluation, the Trypan Blue dye was used to enter cells with compromised membranes, making them appear dark upon bright field imaging. Colony-forming unit (CFU) and fibroblast colony-forming units (CFU-F) were used as an indicator of cell activity. Colony assays using the medium of Methocult™ H4434 Classic (Stemcell Technologies Inc., Vancouver, Canada). Fibroblast colonies were determined using mediums of MesenCult™ MSC Basal and MesenCult™ Stimulatory Supplements (Stemcell Technologies Inc., Vancouver, Canada). Finally, BMAC sample were tested for sterility using BD BACTEC™ 9050 Blood Culture System (Becton, Dickinson and Company, New Jersey, United States).

Data Management and Statistical Analysis

Data were collected, stored, and managed using paper-based case report forms. All data are shown as the mean and the standard deviation. Clinical characteristics of patients were resumed as counts for categorical variables.

RESULTS

All the fractures were grossly stable. The average time from the initial injury to the MSCs implantation was 6.4 months (range, 6 - 11 months). None of the patients presented peri-operative complications that could be attributed to the percutaneous harvesting and reimplanting technique. The average follow-up period was 13.4 months (range, 6 - 32 months). All of 10 delayed or nonunion healed after treatment with percutaneous injection of autologous BMAC. The mean time for new bone formation was 3.3 months (range, 2 - 4 months), for clinical union was 5.2 months (range, 2 - 7 months),

and for radiological union was 11.8 months (range, 11 - 15 months) (Fig. 2. and Fig. 3).

After concentration of bone marrow aspirates, progenitors preparing injections contained a mean ratio of 1.75 ± 0.85 (%) $CD34^+$ cells, a mean concentration of 2.53 ± 0.75 ($\times 10^6$) $CD34^+$ cells/ml, a mean number of 21.14 ± 5.41 ($\times 10^6$) $CD34^+$ cells, and a mean viability of 98.10 ± 1.29 (%) $CD34^+$ cells. Colony-forming unit (CFU) and fibroblast colony-forming units (CFU-F) were detected in all the samples incubated in vitro. The result of all samples of BMAC was negative for bacteria cultures.

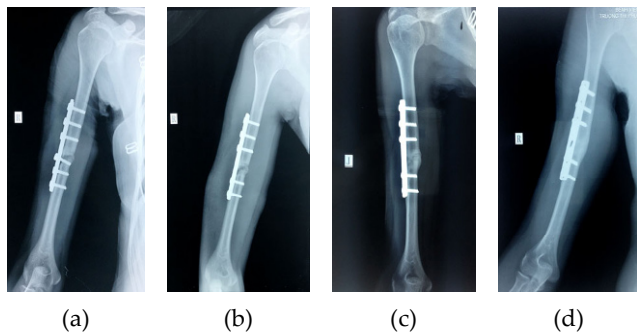


Figure 2. A 29-year-old woman still had a painful non-union of the humerus 8 months after surgery for open reduction and internal fixation by plate. (a) X-ray showed non-healing fracture of humerus at 8 months after surgery; (b) New callus bridging the non-union site on X-ray at one month after percutaneous autologous bone marrow injection and a screw removal at the fracture site; (c) Bone union on X-ray at 4 months after percutaneous injection; (d) X-ray at 12 months after percutaneous treatment showed mature remodeled callus in the area of the graft placement.

DISCUSSION

Our study showed that all 10 patients treated with percutaneous concentrated autologous bone marrow implantation performed significant bone union on their radiographs and good return to normal activities as before the fracture occurred. Healing time after bone marrow injection was significantly shortened. There was no local and systemic complication related to the intervention. Haematoma formation or infection was not found at the position of aspiration. Aspiration of bone marrow from the both iliac crests has not been

a cause for chronic pain.

For the treatment of the delayed or nonunion, mechanical stabilization has been the main technique. All our patients presented with stable delayed or nonunion fractures, which there was no excessive movements at the fracture site. Beside mechanical and biochemical aspects, biological processes played a major role in the causes of delayed or nonunion fracture in recent studies (Bhargava, 2007; Bigham-Sadegh, 2014; Hernigou, 2005b). The rate of bone union was influenced by the cellular proliferation that results in the formation of the callus, the periosteum, and the status of bone marrow in the generation of osteoblasts (Fox, 2014; Hernigou, 2005b; Qin, 2014).

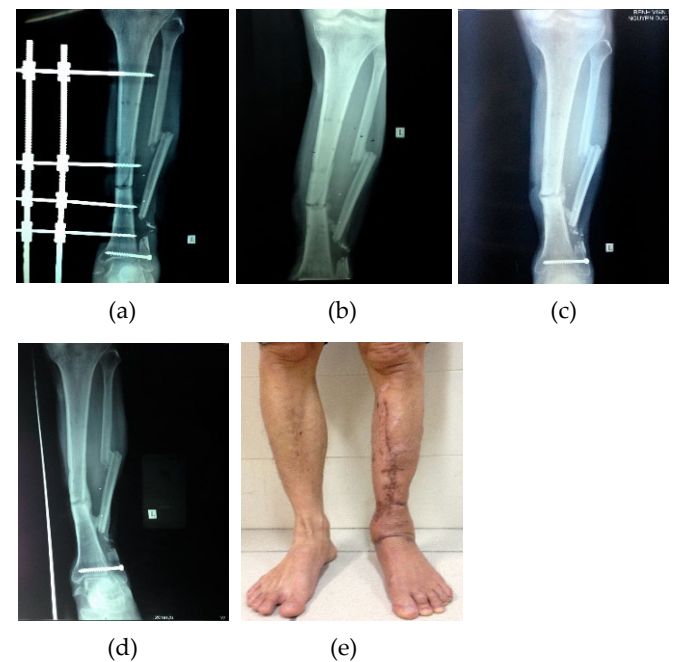


Figure 3. A 40-year-old man had a tibial delayed union 6 months after open fracture. (a) The patient was treated with a debridement and external fixation. (b) The patient had new callus bridging the fracture site 3 months after percutaneous treatment with autologous bone marrow, external device removal and plaster casting. (c) Bone union of the fracture site 6 months after percutaneous injection. (d) X-ray 12 months post injection of bone marrow showed mature remodeled callus in the fracture site. (e) The patient was asymptomatic for weight bearing and resumed normal activities at 12 months follow-up.

Studies of Hernigou et al. showed that deficiency in the population of local connective tissue by progenitors may happen in the cases of previous infection,

tissue defects, and injured vascularity, and this could be the cause of delayed or nonunion. Furthermore, the periosteum that has an osteogenic potential had usually been lost in these cases; consolidation could only be achieved by the osteoblasts at the fracture site. The number of osteoblasts was insufficient, so the proliferative capacity could be lower and bony union could be difficultly achieved (Hernigou, 2005a; Reichert, 2007).

Over the past two decades, progress in the understanding of the role of the biology of bone union had led to the development of other forms of bone grafting. The purpose being to facilitate osteoinduction and osteoconduction, and to lead to osteogenesis (Liebergall. M., 2013; Oryan A., 2014; Shah, 2013; Shenoy, 2014). The harvest of autologous bone marrow has been developed in previous studies (Bielecki, 2006). Since bone marrow contains osteogenic progenitors, the injection of bone marrow is considered to have the potential for effective bone regeneration (Hernigou, 2006; Undale, 2009). The implantation of bone marrow can increase the local biology and optimize the potential of osteoprogenitors (Ohgushi, 2014). On the other hand, autologous bone marrow injection enhances cellular numbers and stimulates the cells to fabricate a structural osteoconductive substrate with extremely low donor site morbidity (Pountos, 2010). Bone marrow injection is a mini-invasive technique and can contribute to bone union in cases of stable delayed or nonunion (Bhutia, 2015; Singh, 2013; Tseng, 2008). It has reduced soft tissue damage, improved the recovery period, and minimized scarring. It is also consistent with the modern treatment trends in the fields of Orthopaedics, especially in difficult situations (Pountos, 2010).

In our studies, biologic scaffolds were not used in any of these procedures and the trocars were not remove the callus or fibrous tissue between bone gaps. Bone marrow was directly injected in the delayed or nonunion gap and around the bones ends. Using trocars to cannulate the fracture site is the mechanism that allows the transformation of fibrous tissue into callus. This may well create enough traumas to make a whole marrow scaffold. Then, the fibrous tissue interposed between the bones ends can be ossified and stop the micro-motion at the delayed or nonunion site in order to allow union of the gap (Centeno C.J., 2011). All of 10 patients in our series healed their fracture.

Despite the success rates of fresh bone marrow aspirates for implantation in recent studies (Hauzeur, 2010; Kassem, 2013; Singh, 2013; Wilkins, 2003), an

apparent correlation was observed between the number of the injected progenitor cells and the bone union rate (Pountos, 2010). The success of whole bone marrow implantation is dependent on the sufficient populations of the transferred progenitors. Therefore, techniques that are able to enhance the volume of osteoprogenitor cells will contribute good clinical outcomes (Hernigou, 2005a). The mean ratio of CD34⁺ cells in bone marrow grafting prior injection in the study of Gangji *et al.* achieved 1.0 ± 0.2 (%) of CD34⁺ cells (Gangji *et al.*). In present study, bone marrow aspirates was concentrated by centrifugation contained a mean ratio of 1.75 ± 0.85 (%) CD34⁺ cells, a mean number of 21.14 ± 5.41 ($\times 10^6$) CD34⁺ cells, and a mean viability of 98.10 ± 1.29 (%) CD34⁺ cells prior to injection. Bacterial cultures of BMAC were completely negative, and all the cultures of the CFU and CFU-F displayed positive staining in vitro. We believe that the clinical application of BMAC is a safe procedure. Hernigou showed that the amount of available oxygen at fracture site might be one of the limiting factors after implantation (Hernigou, 2005a)et. The implanted progenitor cells have to be a competitor with other cells for oxygen. The concentration of bone marrow aspiration helps to exclude all other cells and optimizes progenitor cell survival for bone formation (Pountos, 2010).

Our study has the limitations because the number of patient was limited and a measurement of the number of CFU-F was not performed. Therefore, it is very difficult to allow conclusive judgments. Since the volume of callus formation in this technique is limited, the bone defect gap should be indicated in a limited way.

CONCLUSION

The result of current study is encouraging in the initial outcome. Percutaneous autologous bone marrow implantation could be an effective and safe option for the treatment of delayed or nonunion fractures. The efficiency related to the population of progenitor cells in the concentrated bone marrow aspiration. However, more studies are needed for definitive conclusions. Beside of pure bone marrow implantation, composites of the porous implantable material with concentrated bone marrow will be evaluated for their efficacy in delayed union or nonunion in future application.

Abbreviation

Mesenchymal stem cells (MSCs); bone marrow aspiration concentrate (BMAC); Colony-forming unit (CFU); fibroblast colony-forming unit (CFU-F).

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Competing interests

The authors declare that they have no competing interests.

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