

The effect of autologous concentrated bone-marrow grafting on the healing of femoral shaft non-unions after locked intramedullary nailing

João Antonio Matheus Guimarães^{a,*}, Maria Eugenia L Duarte^a, Marco B Cury Fernandes^a, Verônica F Vianna^a, Tito H N Rocha^a, Danielle C Bonfim^a, Priscila L Casado^a, Isabel C C do Val Guimarães^b, Luis G Coca Velarde^c, Hélio S Dutra^d, Peter V. Giannoudis^e

^a National Institute of Traumatology and Orthopaedics, Rio de Janeiro, Brazil

^b School of Medicine, Fluminense Federal University, Rio de Janeiro, Brazil

^c Epidemiology and Biostatistics, Fluminense Federal University, Rio de Janeiro, Brazil

^d Institute of Health Sciences, Federal University of Rio de Janeiro, Brazil

^e School of Medicine, University of Leeds, Leeds, UK; Leeds Biomedical Research Unit, Chapel Allerton Hospital, Leeds, UK

KEYWORDS

Bone marrow grafting
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Fracture healing
Non-union

ABSTRACT

The aim of this study was to assess the union rates in a series of patients with failed femoral shaft aseptic non-union who were treated with percutaneous concentrated autologous bone marrow grafting. Bone marrow harvesting and cell injection were performed under general anaesthesia in a single surgical procedure. Radiographic union was diagnosed in fractures with a score ≥ 10 according to the radiographic union scale in tibial fractures (RUST) and confirmed by clinical examination. Eight out of 16 patients progressed to consolidation (RUST score ≥ 10). Radiographic evidence of fracture union was observed at an average of 4.75 ± 1.75 months (range 3 to 8 months). All eight patients who did not progress to union within 12 months following the cell grafting procedure had a RUST score ≤ 10 (range 4 to 9). There were no differences in age, number of previous surgeries, duration of non-union and preoperative RUST score between the patients that developed solid union and those with failed consolidation. However, a relationship between the number of osteoprogenitors injected and the rate of union was noted, $20.2 \pm 8.6 \times 10^8$ versus $9.8 \pm 4.3 \times 10^8$, $p < 0.005$, between the patients with and without union, respectively. The efficacy of percutaneous autologous concentrated bone marrow grafting seems to be related to the number of osteoprogenitors available in the aspirates. Optimisation of the aspiration technique and concentration process is of paramount importance to increase the incidence of a successful outcome.

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Introduction

The treatment of non-union is a challenge to orthopaedic surgeons even with the on-going increase in knowledge of the mechanisms of fracture healing [1–7]. In the United States, an estimated 5 to 10% of the 6.2 million fractures that occur annually are associated with an impaired fracture healing response and develop delayed union or non-union [8,9].

All stages of the bone repair process require the interaction of cells and biologically active molecules [10–12]. Mechanical stability and vascular integrity at the fracture site are also important parameters that contribute to the healing response [13]. Excessive motion that causes mechanical instability at the fracture site, and impaired blood supply due to damage to

nutrient vessels, have a direct influence on the development of non-union [13,14]. Giannoudis et al. [15] have also demonstrated a striking correlation between the use of pharmacological agents such as NSAIDs for longer than four weeks, and the incidence of non-union of the femoral diaphysis.

Femoral shaft fractures are common in young adults and result from high-energy trauma caused by motor vehicle accidents, falls from a height and gunshot injuries. The method of choice for fixation of this type of fracture is locked intramedullary nailing with the aim to recover length, alignment, and rotation to the femur [16]. Non-union after femoral nailing was not a common finding in early reports [17]; however, the increased use of intramedullary nailing for the treatment of femoral shaft fractures has been associated with rates of non-union as high as 13.6%, and improved survival of severely injured patients [18]. In a series of 278 patients (280 fresh femoral shaft fractures) treated with intramedullary nailing, more than one-tenth of the patients required additional surgery for failed consolidation [19]. Failed union of the femoral shaft after nailing is a serious complication and is difficult to treat [20,21].

* Corresponding author at: Instituto Nacional de Traumatologia e Ortopedia (INTO), Avenida Brasil 500, São Cristóvão, 20940-070, Rio de Janeiro, RJ, Brazil. Tel.: 55-21-2134-5102; fax: 55-21-2135-5089.

E-mail addresses: jomatheus@uol.com.br, jguimaraes@into.saude.gov.br (J.A. Matheus Guimarães).

Table 1

Summary of clinical details of patients with non-union of femoral shaft fractures treated with intramedullary nailing

	Sex, age	Injury mechanism	Fracture Classification (OTA [28] and G-A [29])	Previous surgeries	Non-union		Comorbidities
					Type	Duration (mo)	
1	F, 35	Car accident	Closed, 32A	2	Oligotrophic	95	None
2	F, 43	Car accident	Closed, 32A	2	Oligotrophic	50	None
3	F, 44	Car accident	Closed 32B, 31B	3	Oligotrophic	71	None
4	M, 27	Motorcycle accident	Closed, 32B	4	Oligotrophic	48	None
5	M, 52	Fall from height	Closed, 32A	3	Oligotrophic	62	None
6	M, 45	Running over	Closed 32C, 31B	1	Oligotrophic	60	None
7	F, 54	Gunshot wound	Open 32B, GII	1	Atrophic	22	None
8	M, 28	Motorcycle accident	Open 32A, GI	3	Oligotrophic	69	None
9	M, 44	Motorcycle accident	Closed 32C, 31 B	1	Atrophic	15	None
10	M, 24	Car accident	Closed 32B	1	Atrophic	9	Tobacco use
11	M, 45	Car accident	Closed 32A, 31B	1	Atrophic	14	Tobacco use/DM
12	M, 37	Motorcycle accident	Open 32B, GI	4	Oligotrophic	97	Tobacco use
13	M, 19	Running over	Closed 32A	1	Atrophic	15	Tobacco use
14	M, 30	Motorcycle accident	Closed 32B	1	Atrophic	15	None
15	F, 19	Motorcycle accident	Closed 32A	2	Atrophic	12	None
16	M, 59	Fall from height	Open 32B, GI	2	Oligotrophic	15	Tobacco use

M, male; F, female; OTA, Orthopaedic Trauma Association; G-A, Gustilo and Anderson classification; mo, months; DM, diabetes mellitus.

Several surgical strategies have been reported for the treatment of aseptic long bone non-union, including exchange nailing [22–25], biological enhancement with autologous iliac crest bone grafting [26,27], implantation of bone morphogenetic proteins (BMPs) [28–31], platelet rich plasma (PRP) [32] and allograft [33].

Hernigou et al. [34] developed a minimally invasive alternative to treat atrophic non-unions of the tibial shaft. Bone marrow aspirates from the iliac crest were sorted on a cell separator and injected in the non-union site. Bone union was obtained in fifty-three out of sixty patients with atrophic uninfected non-union of the tibial shaft, four to sixteen weeks after the procedure. Also, the efficacy of the percutaneous autologous bone marrow grafting seemed to be related to individual factors, mainly the number of osteoprogenitors available in the aspirates from the bone marrow. The literature regarding this method of treatment for femoral shaft non-unions remains obscure.

The purpose of this study, therefore, was to assess the union rates following treatment with percutaneous autologous bone marrow grafting in a series of patients with failed femoral shaft union after treatment with intramedullary nailing.

Patients and methods

From January 2010 to January 2011, patients who were diagnosed in our institution with a femoral non-union and who failed to respond to treatment with intramedullary nailing were eligible to participate in this study. Patients were included if they were aged at least 18 years, had been diagnosed with a femoral shaft aseptic non-union, were not routinely on any medication that could influence the fracture healing response and did not suffer from any systemic inflammatory disease process. Patients were excluded if they were aged under 18 years, had pathological fractures or were lost to follow-up.

Non-union was defined as failure of the fracture to heal within nine months with no radiographic signs of progression of the fracture callus between the third and sixth month after the fracture [35]. Clinical details for the patients in the study are summarised in Table 1.

After the cell grafting procedure, all patients were followed-up by anterior-posterior (AP) and lateral radiograph views at six weeks, four months, six months and 12 months to monitor the

progress of bone formation. Union was diagnosed if on clinical examination there was no tenderness or pain at the fracture site with full weight bearing. Radiographic union was diagnosed in fractures with a score ≥ 10 according to the radiographic union scale in tibial fractures (RUST) [36]. This scoring system takes into account the presence/absence of mature bone bridges crossing the fracture site in AP and lateral radiographic views and the presence/absence of a visible principal fracture line, see Table 2. Patients were considered unresponsive to the cell grafting treatment if they had persistent unconsolidated fracture (score ≤ 10) and clinical limitations due to local pain at the end of the follow-up period.

Bone marrow aspiration and concentration

Bone marrow harvesting and cell injection were performed under general anaesthesia in a single surgical procedure. A needle for bone marrow aspiration (11G $\times$ 10 cm, Bloodline, Medolla, Italy) was inserted by hand into the posterior iliac crest. The needle was regularly reoriented within the iliac crest during the successive aspirations as previously described [37,38] to optimise the number of harvested cells. The marrow samples were harvested in small amounts (2 mL) and the contents of each syringe were pooled in the container of the bone-marrow-collection kit (R4R 2107; Baxter, Deerfield, Illinois) containing anticoagulant solution. The final volume of bone marrow aspirate (200 mL) was then filtered through a sequence of successively smaller-diameter mesh filters provided in the collection kit. The cells were finally collected in a blood transfer pack unit and the nucleated cells were sorted on a cell separator (Sepax, Biosafe, Geneva, Switzerland).

Bone marrow mononuclear cells concentrate

The aspirated material was reduced to a final volume of 40 mL by removing most of the red blood cells and the plasma by centrifugation (35-minute volume reduction cycle). The bone marrow mononuclear cell fraction (BMMNCs), containing both mesenchymal and haematopoietic stem cells, was collected at the end of the centrifugation cycle and constituted most of the cell concentrate that was injected in the non-union site. The number of BMMNCs in the cell concentrate that was ultimately

Table 2
Radiographic union scale in tibial fractures (RUST)

Score per cortex*	Radiographic criteria	
	Callus	Fracture line
1	Absent	Visible
2	Present	Visible
3	Present	Invisible

*The individual cortical scores (anterior, posterior, medial and lateral) are added to provide a RUST value for a set of radiographs of 4 (definitely not healed) to 12 (definitely healed).

injected in the non-union site was counted as follows: the number of nucleated cells / mL $\times$ total volume (40 mL). The time between bone marrow aspiration and grafting at the non-union site was less than ninety minutes.

Bone marrow grafting

A guide wire was positioned into the non-union gap under biplane fluoroscopic control. A cannulised drill was placed over the guide wire for drilling a tunnel. A trocar (8G $\times$ 15 cm, MDL, Delebio, Italy) was inserted using the same path and the cells were then injected into the medial, lateral, posterior and anterior aspects of the non-union site. The same surgeon performed the aspiration and cell injection at all times.

Statistical analysis

Data entry and statistical analysis were implemented with STATA 11.1 (StataCorp, Texas, USA). The sample size included all patients who were diagnosed in our institution with a femoral non-union and who failed to respond to treatment with intramedullary nailing during a one-year period and who met all the inclusion criteria.

The significance of nominal variables between the patients was assessed by the chi-square (χ^2) test. Student's t-tests were used to determine the differences in age, preoperative and postoperative RUST score, and the number of nucleated cells injected in the non-union site. The non-parametric Mann-Whitney test was used to determine the difference between the duration of non-union. Multivariate logistic regression analysis was performed to enable the exploration of several covariates simultaneously. A p value of 0.05 or less was considered statistically significant.

Results

A total of 19 patients with femoral aseptic non-union were treated with injection of BMMNCs during the study period. Three patients were lost to follow-up; therefore, 16 patients (11 male) with a mean age of 37.8 years (range 19–59 years) met the inclusion criteria. All patients were treated with autologous BMMNCs at the National Institute of Traumatology and Orthopaedics in Rio de Janeiro, Brazil. Ethical clearance was obtained from the local Ethics Committee (n°123/06). There were no local or systemic complications related to the cell grafting procedure and all patients were discharged from hospital within 24 hours after the intervention.

Twelve patients had initially sustained a closed fracture and four patients had an open femoral shaft fracture. According to the Gustilo-Anderson classifications of open wounds [39], three patients with open fracture were type I, and one patient was type II. The shaft fractures were classified according to the Orthopaedic Trauma Association (AO-OTA) [40] as type 32-A (n=7), type 32B (n=7), and type 32C (n=2). Four patients had a concomitant

healed fracture in the femoral neck (31B). Prior to the cell grafting procedure, all patients achieved mechanical stabilisation of the fracture site with the insertion of an intramedullary-locking nail. This procedure was the only surgical intervention in seven patients. In the remaining patients, besides the procedure for the insertion of the intramedullary nail, they were submitted to one (n=4), two (n=3), or three (n=2) previous surgical interventions related to the femoral shaft fracture. At the time of cell injection, patients were diagnosed either with atrophic or oligotrophic non-union for a mean time of 41.8 months (range 9–97 months). Five patients had comorbidities: all were tobacco users and in one there was an association with diabetes mellitus (Table 1).

Bone union (score ≥ 10) occurred in eight of the sixteen patients according to the RUST. The postoperative radiographic score in these patients increased significantly ($p < 0.0007$) after the cell grafting at the non-union site. Early bone formation was first viewed between the third and eighth month after bone marrow grafting. Radiographic evidence of fracture union was observed at an average of 5.0 ± 1.75 months (range, 3–8 months). All eight patients who did not progress to union had a radiographic score ≤ 10 (range 4 to 9) according to the RUST 12 months after the cell grafting procedure. Notably, treatment with bone marrow grafting significantly increased ($p < 0.0005$) the local production of bone at the non-union site in patients who did not have a healed fracture.

Individual values for the radiographic RUST scores before and after cell grafting, the number of cells injected and the healing time are displayed in Table 3.

There were no differences in age, number of previous surgeries, duration of non-union and preoperative RUST score between the patients who developed solid union and those with failed consolidation. Nominal variables, such as sex, fracture and non-union types, and tobacco use, also did not differ between the patients with healed non-union and those in whom the treatment with BMMNCs grafting failed.

A multivariate logistic regression analysis of individual parameters was conducted to assess factors concurrently. The initial univariate analysis indicated that the number of BMMNCs is a potential predictive factor for the consolidation of femoral shaft non-union treated with intramedullary nailing (Table 4). Indeed, among the variables that were investigated, the number of transplanted BMMNCs was the variable most relevant to the outcome. The bone marrow grafts used for the eight patients who subsequently had bone union contained a total of $14.61 \pm 7.37 \times 10^8$ BMMNCs. The grafts used in the patients in whom treatment failed contained a significantly lower number of total nucleated cells (mean $9.8 \pm 4.3 \times 10^8$, $p = 0.005$) compared with the patients in whom the non-union consolidated. The total number of BMMNCs from each patient is displayed in Table 3.

Clinical improvement, represented by pain reduction at the fracture site with full weight bearing, was accompanied by radiographic consolidation in all patients who responded to treatment (Figures 1–3). Patients who did not respond to cell treatment were referred to revision surgery consisting of exchange nailing to achieve healing.

Discussion

Long bone non-union remains a difficult clinical problem as it has been shown to be associated with pain, drug and alcohol abuse, prolonged stay off work and family breakdown. This complication may also induce prolonged hospitalisation and secondary interventions with the associated inconvenience and additional costs for the private and public healthcare systems.

The diamond concept has been proposed for the treatment of non-unions: this concept highlights the importance of restoring

Table 3

Radiographic scores according to the RUST before and after cell grafting, number of nucleated cells injected and follow-up

Case	Preoperative RUST score	Total number of nucleated cells ($n^{\circ} \times 10^6$)	Postoperative RUST score*	Healing time (months)
1	4	10.6	8	No union
2	5	7	7	No union
3	5	7.4	9	No union
4	5	34	11	6
5	6	17	10	8
6	6	15.4	12	3
7	4	8	11	4
8	6	12.5	7	No union
9	4	8	4	No union
10	6	22	12	4
11	4	19	12	5
12	4	15	4	No union
13	5	10.3	7	No union
14	4	7.7	7	No union
15	5	23	12	6
16	6	17	12	4

*Postoperative RUST score was determined 12 months after cell grafting.

Table 4

Multivariate logistic regression analysis (reference: united fracture)

Parameter	Z	p-value
Age	-1.15	0.25
Fracture type	1.30	0.19
Number of previous surgery	1.28	0.20
Number of cells	-1.92	0.05

both the mechanical and the biological environment to achieve a successful outcome [13]. Fracture union requires the presence of the factors combined in the diamond concept: an adequate cellular environment, growth factors in sufficient amounts, an adequate bone matrix, mechanical stability and appropriate local vascularisation. Delayed union or non-union occurs in 5% to 30% of the patients who lack one or more of these factors [13].

The current gold standard for the treatment of non-union of the femoral shaft after fixation with intramedullary nailing is exchange nailing, which is defined as the removal of the original intramedullary nail followed by over-reaming and the insertion of a new nail that is larger in diameter and locked proximally and distally [41]. However, the rates of osseous union following a single exchange nailing of an aseptic, non-comminuted femoral shaft non-union have been reported to range from 53% to 100%, see Table 5 [21,42–49].

In the current series of patients, the mechanical environment was assessed by careful evaluation of the radiographs. All patients in the study showed a non-union of the femoral diaphysis treated with interlocking nail. There were no radiological features of aseptic loosening of the metal work, and no screw or nail breakage. To be precise in the area of injection, a cannulised drill was placed over a guide wire for drilling a tunnel that served to create a biological chamber [50] where the cells were delivered. The cells were injected circumferentially at the site of the non-union to ensure they would become available all around the non-union site.

The injection technique for BMMNCs was standardised in this study and mechanical factors were excluded as possible candidates for the non-union; therefore, we hypothesised that the lack of an appropriate biological stimulus should be considered as a major factor for non-union in this series. A strategy was therefore developed to treat patients with non-

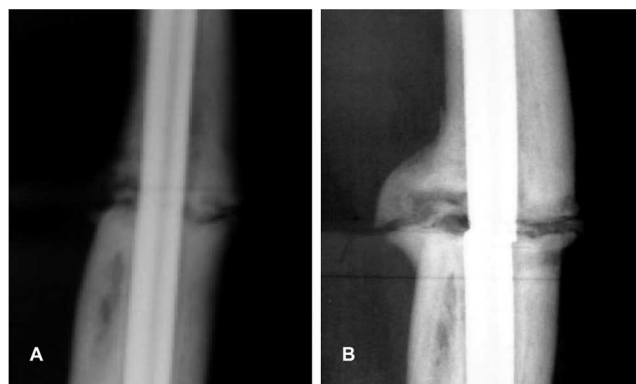


Fig. 1. Radiographs of a forty-four-year-old female (Case 3) who sustained a femoral shaft fracture (32B) in a car accident. (A) Anteroposterior radiograph shows the atrophic non-union site ninety-five months after initial fixation (intramedullary nailing). (B) At the time of the injection of autologous bone marrow, the patient was unable to be fully weight-bearing and complained of severe pain at the non-union site. Although there was bone formation, disturbed consolidation has resulted in a fatigue failure of the nail ten months later.

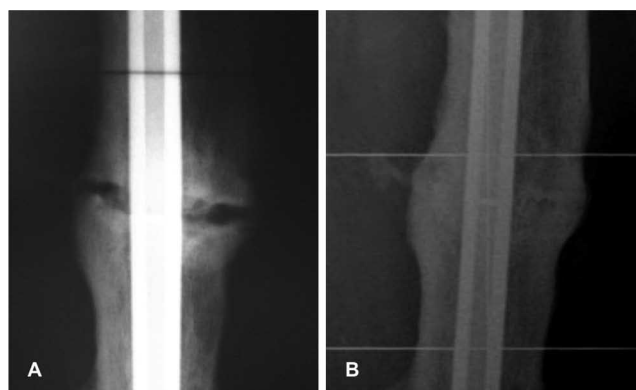


Fig. 2. Radiographs of a forty-five-year-old male (Case 6) who sustained a closed femoral shaft fracture (32C). (A) An anteroposterior postoperative radiograph taken 60 months after static intramedullary nailing shows the evident atrophic non-union. At this time the patient was highly symptomatic. (B) An anteroposterior radiograph taken eight months after BMMNCs grafting shows the healed femoral non-union.

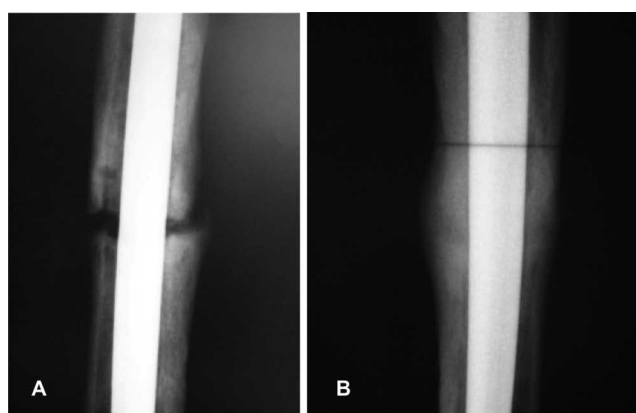


Fig. 3. Radiographs of a nineteen-year-old female (Case 15) who sustained a closed fracture of the femoral shaft (32A). (A) Lateral X-rays of non-union made twelve months after intramedullary nailing. (B) Lateral radiograph taken six months after the injection of 23.0×10^6 BMMNCs, obtained by centrifugation of total bone marrow, shows the healed femoral shaft non-union.

union with a supplementary osteogenic stimulus in the form of an injection at the non-union site of BMMNCs obtained by aspirates from the iliac crest, during a single surgical procedure.

Table 5

Review of the literature on contemporary techniques of exchange nailing for femoral diaphysis

Author	Number of patients	Bone-grafting at time of exchange	Osseous union
Wu and Chen, 1997 [42]	16	0 (0%)	100%
Furlong et al., 1999 [21]	22	12 (48%)	95%
Hak et al., 2000 [43]	23	0 (0%)	78%
Weresh et al., 2000 [44]	19	4 (21%)	53%
Wu and Chen, 2002 [45]	36	0 (0%)	92%
Pihlajamäki et al., 2002 [46]	18	4 (22%)	78%
Banaszkiewicz et al., 2003 [47]	19	0 (0%)	58%
Finkemeier & Chapman, 2002 [48]	39	0 (0%)	74%
Shoeder et al., 2009 [49]	42	0 (0%)	86%

The approach to provide most of the area of the non-union site with an additional contingent of cells with osteogenic differentiation potential resulted in an uneventfully osteoforming response in all patients of this series. However, the magnitude of this response was not uniform among the patients. Eight of the sixteen patients treated with percutaneously implanted BMMNCs showed healing on radiographs and returned to full pre-fracture functional activities. Non-union persisted in the remaining eight cases that required exchange nailing to achieve solid union. The RUST score was a valuable tool for measuring the magnitude of the osteoforming response after the injection of BMMNCs. Overall, the RUST score was increased in 14 out of 16 patients after the cell treatment and only patients with scores equal to or higher than 10 presented clinical and radiographic consolidation (Table 3).

The first report of injectable bone marrow to treat non-union was published in the 1980s [51]. Connolly et al. used relatively large amounts of bone marrow (100–150 mL) to successfully treat eight out ten tibial non-unions [51–52]. Thereafter, bone marrow injections were widely used to treat tibial [53–57], femoral [55,58,59], humeral [53,55,59,60], forearm [53,55,61] and

clavicular [62] non-unions. Results regarding the effectiveness of the procedure are displayed in Table 6. In total, 327 cases of non-unions have been described. From those, 248 (75.7%) were tibial non-unions, 32 (9.8%) were femoral non-unions, 20 (6.1%) were humeral and the rest (27; 8.2%) were non-unions of the radius and ulna. The mean age was 40 years. Excluding the two case reports (one patient in each), the general union rate after treatment with percutaneous injection of autologous bone marrow was 82.4% (288 out of 327 non-unions; range 57–94%). The mean time to achieve solid union was 4.8 months (range 2.5–8.1) (Table 6). However, in only one of the studies [27] the authors report the use of concentrated bone marrow instead of pure total marrow grafting. This retrospective study evaluated 60 patients with non-infected atrophic non-union of the tibia who had undergone percutaneous autologous bone marrow grafting. Similar to the present study, marrow was aspirated from the iliac crest, concentrated in a cell separator and injected into non-union sites. However, Hernigou and co-workers [34] included in their study patients with only six months of evolution of non-union, patients with mechanical instability in the fracture site treated with different forms of stabilisation, such as external fixation and external immobilisation, and fractures localised in different anatomical regions of the tibia. Although the patient population in the current study was smaller, it was a more uniform sample: all patients had non-union for over nine months and all fractures were localised in the same anatomical site in the femur (diaphysis). Furthermore, there was no excessive movement at the fracture line in any of the patients because of the presence of the interlocking intramedullary nail, which was not associated with any signs of osteolysis (indicative of mechanical instability).

The following possible causes of treatment failure were not the cause in the current case series: fracture duration, the nature of the fracture (open or closed), the extent of the initial osteogenic response at the fracture site determined by the RUST, and the number of previous surgeries. The only detected difference between patients whose fracture healed and those for whom it failed to unite was the number of BMMNCs injected at the non-union site ($20.2 \pm 8.6 \times 10^8$ versus $9.8 \pm 4.3 \times 10^8$, $p < 0.005$).

Table 6

The effectiveness of bone marrow (BM) injections for non-unions

First author/year	Number of non-unions	Type	Mean age (y), n (range)	United (n) (%)	Time to union (months, n (range))	Comments
Connolly/1991 [52]	10	Tibia 10	30 (18–82)	8(80)	6.7 (5–10)	Injection 100–150 mL of total BM Cast immobilisation
Garg/1993 [53]	20	Tibia 15 Humerus 3 Ulna 2	35 (18–65)	17(85)	5 (3–7)	Injection 15–20 mL of total BM
Sim/1993 [60]	11	Tibia 8 Femur 1 Humerus 1 Ulna 1	38 (19–62)	9(81)	2.5 (1–5.75)	Injection 50–200 mL of total BM
Matsuda/1998 [58]	7	Femur 7	53 (24–70)	4(57)	(5–9)	Injection 150 mL of total BM
Sebecic/1999 [54]	Case report	Tibia 1	44	1(100)	5	Injection 150 mL of total BM External fixation
Siwach/2001 [55]	72	Tibia 42 Femur 8 Humerus 12 Forearm 10	41 (26–56)	68(94)	-	Injection 30 mL of total BM. Procedure was repeated where required after 4–6 weeks
Wang/2001 [56]	56	Tibia 56	19–72	53(94)	-	Total BM injection. Procedure was repeated monthly 2–3 times
Wilkins/2003 [59]	69	Tibia 36 Femur 16 Humerus 4 Other 13	42 (15–81)	61(88)	8.1(2–36)	Total bone marrow combined with demineralised bone matrix
Goel/2005 [57]	20	Tibia 20	37 (24–60)	15(75)	3.5(1.5–5.5)	Injection 15mL of total BM.
Hernigou/2005 [34]	60	Tibia 60	40 (18–78)	53(88)	3(1–4)	Injection 20mL of concentrated BM.
Tetreault/2007 [62]	Case report	Clavicle 1	39	1(100)	Within 12	Injection 10mL of total BM.

A relationship between the number of osteoprogenitors injected and the rate of union has been observed previously. In their pioneering report, Hernigou et al. [34], demonstrated a definite relationship between the number of osteoprogenitors injected and the rate of union. They also speculated that the concentrated bone marrow included stem cells as well as other mononuclear cells that exhibit osteogenic or angiogenic properties, which affect the clinical outcome.

Individual differences in the cell composition of the bone marrow have been reported previously. Several variables such as age, sex and local and systemic diseases [63,64–69] have been shown to influence bone marrow cellularity. A large inter-donor variation in the number of nucleated cells and in the multi-lineage differentiation potential of less committed progenitors has also been reported in normal bone marrow [70,71].

Although the current study is limited in terms of the size of the patient population, the findings highlight the paramount importance of the biological environment in bone fracture repair. The authors believe that the effect exerted by the improvement of the biological environment was real and it is likely to be secondary to a sustainable stimulation of the local biology. The treatment of femoral non-union with autologous BMNCs delivered percutaneously to the fracture site via a trocar is a considerably less invasive procedure than many surgical options to treat uninfected femoral shaft non-union, including exchange nailing, which is the current gold standard of treatment. Although union was seen here in eight of the 16 treated patients, a larger-scale randomised clinical study is required to further investigate this treatment approach and to confirm our hypothesis. Another challenge for future studies will be the necessity to standardise the number of nucleated cells injected in the area of the fracture, taking into account the large individual variation in the number of osteoprogenitors that are recovered in the BMNCs fraction.

Conclusions

The efficacy of percutaneous autologous concentrated bone marrow grafting seems to be related to the number of osteoprogenitors available in the aspirates. Optimisation of the aspiration technique and concentration process is of paramount importance to increase the incidence of a successful outcome.

Conflict of interest statement

The authors declare that there are no conflicts of interests with the trademarks included in the manuscript. All authors also disclose no personal, financial or non-financial competing interests.

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