AUTOLOGOUS MARROW INJECTION IN THE TREATMENT OF DELAYED AND NON-UNION IN LONG BONES

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ABSTRACT

A retrospective study of the use of autologous bone marrow injection for the treatment of delayed and non-union of long bones in an Orthopaedic Department, Singapore General Hospital from 1990 - 1991 is presented.

There were 10 patients with 11 fractures (8 tibia and one each of humerus, femur and radius-ulna) available for study. Percutaneous injection of autologous marrow alone was used to stimulate healing of delayed and non-unions treated initially by plating, external fixation and in one case, by plaster cast. Marrow injection stimulated a callus formation sufficient to unite 9 of the fractures. The median time to clinical union was 10 weeks (range 4-23 weeks) and radiological union 17 weeks (range 9-29 weeks). Most patients had discomfort at the donor and injected sites for one to two days. There was one case of infection but none of the significant donor site morbidity was associated with standard open autologous grafting.

Bone marrow injection was effective in stimulating bony union, with numerous advantages and considerably lower morbidity compared with standard open autologous grafting. Shorter inpatient stay was a significant feature.

Keywords : bone marrow transplant, percutaneous injection, non-union, delayed union, bone graft

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INTRODUCTION

Even excluding the case described in the book of Genesis Chapter 2, bone grafting has a very ancient lineage. Sporadic use of bone grafts has been described in the oldest medical records, referred to in myths and legends, and depicted in religious art that is centuries old[1]. Since the first recorded bone graft attempt by the Dutch surgeon Job Van Meek'ren in 1668[2], bone grafting has become one of the most frequently performed orthopaedic procedures. Despite advances in operative fixation techniques and the consideration of many substances for use as implant or transplant materials, fresh autologous cancellous bone remains the most effective graft material for most clinical situations, and the method of open harvesting from the pelvis and operative implant at the fracture site remains largely unchanged since the classic work of Phemister in the 1930s[3].

Three basic mechanisms underlie bone regeneration: osteogenesis, osteoinduction and osteoconduction[4]. Osteogenesis is carried out by pre-existing undifferentiated bone-forming cells. Autogenic bone and marrow grafts are examples of transplants with osteogenic potential. Osteoinduction entails differentiation of uncommitted connective tissue cells into osteoblasts in the presence of an induction stimulus. Osteoconductive grafts provide a scaffolding for the ingrowth of vessels and new bone by facilitating migration of bone-forming components. Goujon[5] first observed that red marrow transplanted to a heterotopic site forms bone. Burwell[6 8] used the osteogenic potential of autologous red marrow to create osteogenesis in allogenic cancellous grafts. Other workers have, in the last 25 years, used autografted marrow to provide osteogenesis in autografts[9], allografts[6-8,10] and xenografts[11]. Bone marrow has also been used in combination with osteoinductive materials such as bone morphogenetic protein (BMP)[12], demineralised bone matrix[13], and as composite grafts with bioceramics[14].

Connolly et al[15] described their experiences with 20 ununited tibial fractures treated by percutaneous marrow injection, as a substitute for standard autologous bone grafting, in conjunction with cast immobilisation and intramedullary nail fixation.

The purpose of this paper is to present our local experience with percutaneous injection of autologous marrow alone in the treatment of delayed and non-unions treated previously by plating, external fixation or cast immobilisation.

MATERIALS AND METHODS

All patients in the Department who received marrow injection between 1990 and 1991 were identified and their case records and X-rays were reviewed.

The following definitions were used:

	Clinical	Radiological
Delayed union	Time to unite unduly prolonged, in excess of prediction by Perkin's timetable[16]. Pain and tenderness when stressed	Fracture site clearly visible, ends not sclerosed
Non-union[17]	Movement elicited at fracture site, painless unless excessive	Fracture site visible, ends may be sclerosed and hypertrophic or atrophic

Clinical union[18] - Absence of mobility between fragments, tenderness on firm palpation and pain on angulation stress.

Radiological union[18] - Visible callus bridging the fracture and blending with both fragments or continuity of bone

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Fig 1 - Multiple needle stick sites marked on the posterior iliac crest from which bone marrow is harvested.

Fig 2 - Injection of bone marrow into the fracture site under fluoroscopic control.

trabeculae across the fracture.

The bone marrow was harvested in 5 ml aliquots from the posterior iliac wing with the patient placed prone and under general or spinal anaesthesia. Multiple needle sticks (Fig 1) were required to harvest volumes of 50-200 ml which were kept fluid by 0.5 ml of heparin. Cell counts were not routinely done but in one case, the presence of mesenchymal cells was confirmed by cytological examination. The marrow was then injected into the fracture site under fluoroscopic control (Fig 2).

Follow-up clinical and radiological examinations were performed at 2 weeks post-injection and subsequently at intervals varying from 1 to 3 months.

RESULTS
During the period of study, there were 10 patients with 11 fractures injected and followed-up till union or otherwise. They are detailed in Table I. There were 9 males and one female; their ages ranging from 19 to 62 years, with a median of 38 years. There were 8 tibial fractures (7 open, of which 4 were Type II and 3 were Type III, and one closed) and one each of humerus (closed), femur (closed) and radius-ulna (open). The

time from injury to marrow grafting ranged from 4 to 30 weeks, with a median of 17 weeks.

Table II gives the treatment details and outcomes of marrow injection. The average duration of hospital stay was 2.4 days, ranging from outpatient procedure to 4 days. Nine of the 11 fractures healed with the technique of marrow injection, the median time to callus formation being 7 weeks (range 2-13 weeks), clinical union 10 weeks (range 4-23 weeks) and radiological union 17 weeks (range 9-29 weeks).

The initial injury, pre-injection, early post-injection and radiologically united roentgenograms of Cases 2 and 8 are presented in Fig 3 and 4.

Most patients had some discomfort and pain at the donor and injected sites for one to two days, requiring only simple analgesics. One patient had post-operative fever which settled with antipyretic, with no sequela. There was one case of post-injection infection.

The outcome of complications and failures are presented as case reports.

Table I - Clinical details of the 10 patients who received bone marrow injection

Case	Sex/Age	Site/type of fracture	Initial treatment	Time from injury (weeks)	State of union/bone gap
1. OWC	M/52	Humerus, mid-distal third Closed, comminuted	Plate	16	Delayed union/5mm gap
2. TTC	M/19	Tibia, mid-distal third Open Grade II	Plate	17	Delayed union/no gap
3. CS	M/38	Tibia, mid-distal third Open Grade II	External fixator	25	Non-union/2mm gap
4. RBY	F/23	Femur, midshaft Closed, comminuted	Plate	4	-/1cm gap under fragment
5. LGH	M/62	Tibia, midshaft Open Grade II	Plate	17	delayed union/no gap
6. YMF	M/19	Tibia, midshaft Closed, spiral	Plaster cast	9.5	Delayed union/4 mm gap
7. OTM	M/36	Radius-ulna, mid-distal third, Open	Plate	19	Non-union/no gap
8. TAS	M/39	Tibia, middle third Open Grade IIIB	External fixator	30	Non-union/gap under fragment
9. NKS	M/45	(R) Tibia, Open Grade II (L) Tibia, Open Grade IIIB	Plate External fixator,	9	-/2cm gap -/3cm gap
10. TTE	M/42	Tibia, mid-distal third Open Grade IIIA	External Fixator	18	Delayed union/1cm gap

413

Table II - Treatment details and followed-up after bone marrow injection

Case	Duration of stay (days)	Volume injected (ml)	Time of callus formation (wk)	Time of clinical union (wk)	Time of radiological union (wk)	Remarks
1. OWC	2	100	3	6	9	
2. TTC	Day surgery	50	8	23	29	
3. CS	4	120	2	15	15	Fever
4. RBY	2	200	11	11	17	
5. LGH	3	120	9	9	19	Chronic osteomyelitis
6. YMF	1	110	2	7	13	
7. OTM	2	30 - radius] 10 - ulna]	4	4	12	
8. TAS	3	120	6	10	19	Mesenchymal cells seen
9. NKS	3	140 (R)	-	-	-	Infection.Open grafting at 29wk
		100 (L)	10	15	19	
10. TTE	3	80	13	-	-	Plate and open grafting at 20wk

CASE REPORTS

Case 5

A 62-year-old man who sustained an open Grade II tibial fracture was initially treated by wound debridement, plating and delayed primary closure. Marrow injection was performed 17 weeks post-injury for delayed union. Radiological union was documented at 19 weeks post-injection and at 12 months, the healing was sufficient to recommend removal of implant. At the time of removal, the fracture was united but there was pus and granulation tissue with loosening of the implant. He was treated with drainage and insertion of gentamicin beads. Fourteen months later, he required sequestrectomy for chronic osteomyelitis.

Case 9

A 45-year-old man sustained bilateral open comminuted tibia fractures, (L) Grade IIIB and (R) Grade II which were treated with external fixation and plating respectively. Nine weeks post-injury, marrow injection was performed prophylactically to hasten union. This achieved union on the (L) in 19 weeks but the (R) required removal of plate and conversion to external fixation for osteomyelitis detected 10 weeks post-injection. Debridement and soft tissue coverage with a free gracilis flap were required. When the infection cleared 29 weeks post-injection, standard open grafting was performed with cast immobilisation. He is still on follow-up.

Case 10

A 42-year-old man was treated by marrow injection 18 weeks after sustaining an open Grade IIIA tibial fracture that was externally fixed. Although there was some callus at 13 weeks, this was inadequate for union. He underwent plating and open grafting 20 weeks post-injection and achieved clinical union in 12 weeks.

DISCUSSION

The reported results from operative bone grafting indicate successful union ranging from 70% to 95%, depending on the nature and location of the non-union and the technique of grafting[19]. Currently the ideal graft is freshly harvested autologous cancellous bone[20], usually taken from the iliac crest, which provides living osteoblasts and bone morphogenetic protein. Its use however, is hindered by morbidity at the donor site, which includes pain, haemorrhage, haematoma, infection, scarring, buttock anaesthesia, muscle herniation, meralgia paresthetica, fracture, subluxation of the hip, gait disturbances and prolonged hospital stay[19,21,22]. The need to open the non-union site also adds to the risk of infection and devascularisation of the fracture where healing is already impaired. In Case 5 of our series, it is unlikely that infection was introduced by the marrow injection but that there was a low grade osteomyelitis right from the start that had been undetected. In Case 9, infection was detected 10 weeks post-injection but we are not certain if this was procedure-related or due to previously undetected osteomyelitis. Notwithstanding that, the use of percutaneous autologous bone marrow injection has numerous advantages and considerably lower morbidity compared with standard open autologous grafting. It is a relatively simple technique that can be done on an outpatient basis and is therefore more cost effective as the inpatient stay is shorter. There is none of the significant complications at the donor and recipient sites, which should encourage early prophylactic grafting in the treatment of high-energy fractures. This may minimise the time to healing.

Marrow injection, while comparing favourably with the healing intervals reported for standard open grafting, however, does not promote union any more effectively nor rapidly than would open grafting[23]. The capacity of red marrow to form bone depends on defined stromal cells in the marrow as the source of osteoblasts after transplantation. Takagi and Urist[12] have shown that bovine bone morphogenetic protein (BMP) used in a composite graft with marrow produces five times more bone than marrow alone, with union rates of 10/10, 2/5 and 0/5 for composite graft, autologous cortical bone, and autologous marrow alone respectively. They concluded that marrow stroma contains preexisting osteogenic precursor cells and mesenchymal cells capable of responding to BMP by differentiating into osteoblasts, ie the determined osteogenic precursor cells (DOPCs) and the inducible osteogenic precursor cells (IOPCs) respectively. The DOPC is located only on bone surfaces and in marrow stroma, behaving as a stem cell capable of both self-replication and differentiation into osteoblasts in direct response to injury or transplantation, independent of inducing agents. The IOPC is present in red marrow, the connective tissue framework of many tissues and in circulating blood, and behaves as a mesenchymal cell capable of differentiation only in the presence of an inducing agent[24]. Marrow in repair may have a dichotomy in differentiation: under optimal conditions to become osteogenic and in adversity to become phagocytic, explaining why infection frequently delays union[9].

Our experience shows that while there is substantial
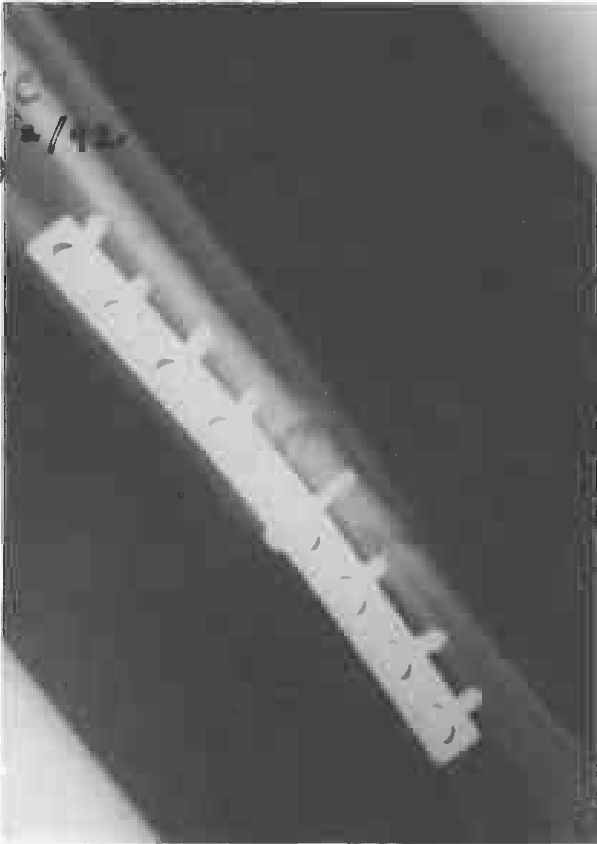
Fig 3 - Roentgenograms of Case 2 (TTC)

3(a) - Initial injury
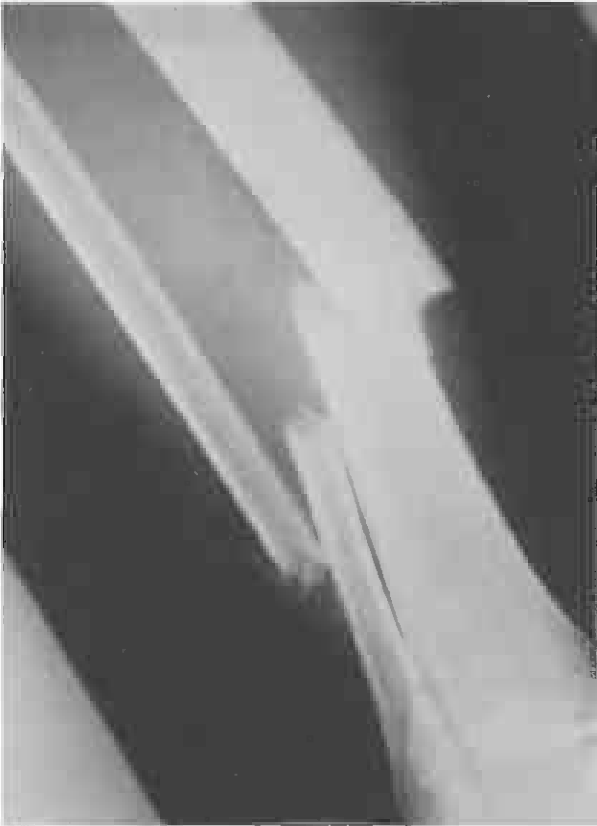
3(b) - Delayed union at 17 weeks post-injury

3(c) - Callus formation at 8 weeks post-injection

3(d) - Radiological union at 29 weeks post-injection

Fig 4 - Roentgenograms of Case 8 (TAS)

4(a) - Initial injury

4(b) - Non-union at 30 weeks post-injury

4(c) - Early post-injection at 6 weeks

4(d) - Radiological union at 19 weeks post-injection

osteogenic potential in autologous marrow, the presence of a bone gap, greater than perhaps 1 cm or so, would require the use of a defect-filling, osteoconductive component, eg bone grafts.

The technique of marrow aspiration and injection was based on that used by haematologists and oncologists for bone marrow transplants[25]. To improve the results of marrow injection, some refinement of technique is in line. Marrow should be aspirated in 3- to 5-ml aliquots by sudden retraction of the plunger of the syringe with simultaneous rotation of the needle to avoid back-filling by venous blood. To reduce clumping, cell aggregates are dispersed by passing the aspirated marrow sequentially through 19- and 22-gauge needles. Smears have shown changes in the staining properties of marrow cells suggesting damage when marrow aspirate is mixed with heparin[11]. It is therefore better to inject the marrow through another marrow needle inserted simultaneously into the fracture site, preferably into the well-vascularised region of muscle attachments. The eventual clotting might even help to retain the marrow within the injected site. Before injection, the marrow needle could be rotated vigorously to break up any fibrous union which would hinder the process of injection. Connolly et al[26] demonstrated that the osteogenetic capacity of marrow is related to cell density and that concentration of marrow by simple centrifugation can enhance the rate and amount of bone formation.

Percutaneous injection of autologous marrow is by no means the sole solution in the treatment of delayed and non-unions. The ideal substitute to autologous cancellous bone graft would have the advantage of mechanical strength combined with bone forming capacity through the presence of living osteogenic cells and bone morphogenetic proteins. One possible approach would be to use cultured autologous osteoblasts to enhance the osteogenic potential of demineralised bone grafts[27]. While our series is small, our results suggest that percutaneous injection of autologous marrow provides cellular stimulation of bone union in fractures with gaps of less than 1 cm. Moreover, it can be performed without the potential donor site morbidity and the need to open the fracture site that is attendant of standard open grafting. The shorter inpatient stay is also a significant feature.

ACKNOWLEDGMENT

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