■ ASPECTS OF CURRENT MANAGEMENT

The use of percutaneous autologous bone marrow transplantation in nonunion and avascular necrosis of bone

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Bone marrow and orthopaedic surgery

During the development of normal bone in the young child, osteoblasts and then haematopoietic stem cells progressively colonise the cartilaginous matrix, resulting in its ossification to bone matrix.[1] It is at this time that red bone marrow appears in the skeleton, starting distally in the foot and hand at around one year of age. In normal children, the marrow in all bone cavities and the spongiosa at the epiphysis is red, because the amount of medullary space in young children is limited due to an abundance of cartilage and the thickness of spongy bone, while the haematopoietic requirements are high due to the growth-related expansion of the blood volume. At the end of the growth period, in late adolescence, the need for haematopoietically active marrow stabilises and adipose involution occurs in many areas of the medulla and spongiosa. Haematopoietic cells are replaced by fat, giving rise to yellow, inactive marrow, but red marrow still persists in certain areas such as the iliac crests. The adult skeleton possesses two types of bone marrow, one red and haematopoietically active, the other yellow due to adipose involution. The haematopoietic stem cell in bone marrow has been studied extensively because of its clinical relevance in therapeutic transplantation of bone marrow. Haematopoietic stem cells are pluripotent and capable of producing progeny that can differentiate into any and all of the cells of circulating blood and the immune system through a well-defined series of steps leading to differentiation into mature blood cells.

In addition to the haematopoietic element, red marrow also contains a stroma where the osteogenic precursor cells are found. The osteogenic capacity of bone marrow was first demonstrated in rabbits by Goujon[2] in 1869. This capacity has been exploited by several authors to reinforce the osteogenic properties of allografts,[3] xenografts[4] and composite grafts[5] by mixing bone marrow removed during the operation with the bone graft.

Burwell[3] showed that primitive osteogenic cells in bone marrow are responsible for much of the biological efficacy of cancellous bone grafts. Friedenstein et al[6] showed that new bone was formed by proliferative fibroblast-like marrow cells which persisted *in vitro* after haematopoietic cells had died and that the number of cells able to proliferate rapidly could be assayed by counting the number of fibroblastic colony-forming units (CFU-Fs) in bone marrow. Several studies[7-13] have shown that at least some of these cells are pluripotent and can differentiate into osteoblasts, chondrocytes, adipocytes, myoblasts and haematopoiesis-supporting cells. Many names have been used to describe the fibroblastic colony-forming cells that can be grown from bone marrow, periosteum, bone fragments, or callus digested from trabecular bone. In this review the term 'tissue progenitors' is used to describe the connective proliferative cell population that can be harvested from bone marrow and which is capable of differentiating into one or more connective tissue phenotypes.

Because bone marrow is known to contain osteogenic progenitors, its implantation was perceived to have the potential to lead to effective bone regeneration. Various preclinical investigations,[14,15] and clinical studies,[15-19] have confirmed this. In clinical practice, autologous marrow is harvested from the iliac crest and immediately transplanted to the site in need of skeletal repair. This type of marrow transfer, or grafting, is a relatively simple procedure which is inexpensive and can be done on an outpatient basis. This technique of transplanting autogenous connective tissue progenitors is the first of the four major cell-based strategies of tissue-engineering which will develop in the future. These are:

1) transplantation of culture-expanded or modified connective tissue progenitors;

2) targeting local connective tissue progenitors with growth factors where new tissue is required;

3) transplanting autogenous connective tissue progenitors;

4) transplanting fully-formed tissue generated *in vitro*.

Grafting with autologous bone marrow in nonunion

Bone is unique in that its ability to regenerate is more predictable than any other tissue in the body. The healing of fractures is the most common condition in which bone regenerates and is usually considered to be biologically easy to obtain with only a few progressing to nonunion. However, although most bony injuries heal without problems, there are occasions when enhancement of the process of repair would be of great benefit to ensure the rapid restoration of skeletal function. It is in these settings that autologous bone marrow transplantation could have a great impact.

The relationship between bony union and bone marrow

Some of the cells of the callus originate in bone marrow. Bony union is a complex phenomenon bringing together many different processes. At first, osteoblasts appear, then callus and finally complete bone tissue is regenerated without any scarring. Previous studies have focused on mechanical, biochemical, histochemical and histological aspects of pseudarthrosis. Recent studies have revealed the timing of the cellular proliferation which results in the formation of the osteoblasts and callus, and the importance of the periosteum[20] and of the bone marrow[21] in the generation of osteoblasts.[22-25] It has been shown by electron microscopy, that bone marrow cells are responsible for the formation of part of the bony callus.[24,25]

The rate of union is influenced by the status of bone marrow of the patient. The relationship between consolidation of fractures and changes in the bone marrow was observed by Ilizarov.[26] He demonstrated that a 1% loss of blood volume induced accelerated consolidation of an osteotomy in rabbits.[26] In the same study he also showed that, following the loss of blood, there was hyperactive haematopoiesis in the bone marrow of the iliac crest.[26] This suggested a link between this haematopoietic activity and the osteogenesis around the osteotomy. Similar observations have been made in experiments in the rat[3,27,28] and the rabbit.[29] This latter paper hypothesised that the link between osteogenesis at the osteotomy and the loss of blood could be a growth factor that he called osteopoietin, whose secretion would be induced by blood loss. This study also showed that if the bone marrow of the iliac crest was more highly populated with blood cells, consolidation of a fracture was more rapid. This phenomenon has yet to be demonstrated in humans.

Several studies have shown that consolidation of a fracture is often delayed in heavy smokers and drinkers. Experiments in animals showed that nicotine had an adverse effect on consolidation.[30] Studies on bone marrow have shown that there is a significant adipose involution of the bone marrow in heavy smokers and drinkers and therefore a potential decrease in the number of progenitor cells.[31] Patients with a history of chemotherapy, alcoholic intoxication and smoking have been shown to have abnormally low levels of progenitors in their iliac crests.[32] Thus, difficulties in consolidation of a fracture may be linked to an overall reduction in the numbers of progenitor cells in the bone marrow as a result of some general physiological problem.

Decrease of progenitor cells at the site of atrophic nonunion. Marrow samples taken from some sites of nonunion in the tibia have shown that in adults, after *in vitro* cloning of the marrow, the number of progenitors is very low compared with the tibia of normal patients.[33] This deficiency in the number of local connective tissue progenitors may occur at the sites of previous trauma, infection, tissue defects, scars and compromised vascularity, as can be observed in nonunion. This suggests that normal tissue repair may be limited by the decreased population of progenitors in local tissues. If the cells in the osteogenic layer of the periosteum and endosteum do not have osteogenic potential, consolidation can only occur by the osteoblasts present at the fracture site. The osteoblasts are not numerous, have a low proliferative capacity and a short half-life of two to three months, which explains the difficulty in achieving bony union.

Grafting with autologous bone marrow can obtain healing of nonunions. Autogenous open cancellous bone-grafts from the iliac crest have long been the most prevalent and effective method of cell transplantation, although only a small fraction of the cells can survive. In the past decade, several clinical studies[15-18,32,34] have demonstrated that transplantation of connective tissue progenitors in aspirated bone marrow can provide bone healing in nonunion. Connolly[34] found bone marrow injection to be successful in the treatment of tibial nonunions. This strategy has been supported by other investigators and many surgeons now use bone marrow because of its biological value and low risk.

In our experience,[33] percutaneous autologous bone marrow grafting is an effective and safe method of treatment of non-infected atrophic nonunion. Like all techniques, it has its limitations. It cannot be used when there are pre-existing angular deformities or prior shortening which require direct access to the nonunion, making percutaneous injection of the bone marrow impossible. As the volume of callus obtainable using this technique is limited, the gap between and displacement of the fragments should be limited. The technique is safe. In our series, there has been no haematoma formation, infection or chronic pain at the site of aspiration. Aspiration of bone marrow from the iliac crest has not been a factor limiting rehabilitation or a cause for a delay in discharge from hospital.

Resection of the scar and fibrous tissue associated with mechanical stabilisation has been thought to be essential for the treatment of atrophic nonunion. It is difficult to

898 P. HERNIGOU, A. POIGNARD, O. MANICOM, G. MATHIEU, H. ROUARD

know the exact mechanism which allows the transformation of fibrous tissue into callus. Bone marrow is injected both into the gap in the nonunion and around the bones. We do not know whether injection of progenitors into the fibrous tissue of the gap can change the properties of this tissue to form bone or if the interposed tissue was stabilised when the bridging callus obtained from the graft around the bone stopped micromotion at the site of nonunion and allowed union to follow. However, this option of cell-based tools highlights a transition from the historical approach including removal of fibrous tissue to a bio-active cell stimulus.

Grafting with autologous bone marrow in osteonecrosis

The relationship between bone marrow and osteonecrosis. The relationships between bone marrow and osteonecrosis of the proximal femur are complex.[35,36] Changes in the bone marrow signal are observed on MRI scans of patients with osteonecrosis.[37] There is an increase in the amount of the fatty marrow in the intertrochanteric portion of osteonecrotic hips. A decrease of osteogenic stem cells is present in the bone marrow of some of these patients.[38]

Steroids have been shown to produce adipogenesis and to stimulate fat-specific genes in cloned bone-marrow cells.[39] In patients with osteonecrosis, abnormalities have been demonstrated in the bone marrow of the iliac crest, following corticosteroid therapy, with a decrease in the stem-cell pool.[38] Cui, Wang and Dalion[40] demonstrated *in vitro* the effect of steroids on cloned bone-marrow cells, with the production of adipogenesis and the stimulation of fat-specific genes. Adipocytes and osteoblasts share a common pool of stem cells and since steroids stimulate the differentiation of marrow stem cells into adipocytes, a decrease in the stem-cell pool may result in the number of pluripotential mesenchymal stem cells being insufficient to provide enough osteoblasts to meet the needs of bone remodelling. This may lead to bone necrosis.

Osteonecrosis is associated with a decrease in progenitor cells in the proximal femur. During normal fetal development, bone marrow has an entirely active haematopoietic function. The process of conversion from red to yellow marrow continues until 25 years of age when the adult pattern of distribution is reached. Normally, in the adult, haematopoietic marrow is absent in the femoral head but red marrow persists in the proximal shaft of the femur. This was demonstrated by Hashimoto[41] as early as 1960 and confirmed by studies using bone-marrow-seeking radiopharmaceutical agents and by MRI.

The distribution of haematopoietic marrow in the proximal femur is related to various factors and may vary. MRI studies have indicated that the conversion of red to fatty marrow occurs prematurely in some patients with avascular necrosis at the upper end of the femur.[37] As a consequence, intramedullary vascularity is altered and this may be a predisposing factor for osteonecrosis since changes in

the bone marrow and bone remodelling are linked. Another consequence is the lack of osteogenic cells, which could influence two different events in the pathogenesis of osteonecrosis, the occurrence of osteonecrosis itself and the bone repair which occurs after osteonecrosis. A decrease in osteogenic stem cells in the femoral head has been observed beneath the sequestrum and in the intertrochanteric region.[42] This has also been confirmed by the observation of the extent of osteocyte death in the proximal femur seen in patients having total hip replacement for osteonecrosis.[43]

Grafting with autologous bone marrow can lead to repair in osteonecrosis of the hip. Reconstruction and repair has been observed after core decompression, but is usually incomplete. One of the reasons of insufficient creeping substitution to achieve bone remodelling after osteonecrosis in the femoral head may be the small number of progenitor cells present in the femoral head in these patients. Although both research and clinical studies have shown that the dead bone may be replaced by living bone, the osteogenic potential for repair is low in osteonecrosis. The number of bone progenitor cells in the uninvolved part of the femoral head and in the trochanteric region is less than in healthy subjects.[42] Whether this decrease is a cause or a consequence of the necrosis is not known. However, because of this lack of progenitor cells, treatment should stimulate and influence bone remodelling by creeping substitution to preserve the integrity of the femoral head. Using progenitor cells or growth factors may be one means of achieving this. It therefore seems sensible to not only use core decompression but also to introduce new cells.

Autologous bone marrow transplantation was proposed for the treatment of osteonecrosis in 1990[44] and good results have been obtained.[45-49] The effectiveness of bone-marrow mononuclear cells may be related to the availability of stem cells endowed with osteogenic properties arising from an increase in the supply of such cells to the femoral head through bone-marrow implantation. Another possible explanation for the therapeutic effect of bone-marrow implantation is that injected marrow stromal cells secrete angiogenic cytokines, resulting in increased angiogenesis and subsequent improvement in osteogenesis.[50]

The technique of marrow aspiration. Bone marrow can be aspirated under general anaesthesia, from the anterior or posterior iliac crests. Prone, lateral or supine positioning of the patient during surgery allows access to the anterior or posterior crests.[32,48] Using a lateral approach, the needle for aspiration may only be advanced into the outer iliac surface for 5 mm, according to the thickness of the iliac crest. The approach through the anterior or posterior iliac crest allows separate aspirations to be obtained by advancing the needle between and parallel to the inner and outer tables. After deep insertion of a bevelled needle 6 to 8 cm long and 1.5 mm in internal diameter into spongy bone,[51] the marrow is aspirated into a 10-ml plastic syringe. At a given depth, the needle is turned 45° during successive aspirations to re-orientate the bevel, thereby affording aspiration

THE JOURNAL OF BONE AND JOINT SURGERY

through 360˚. After one full turn, the needle is moved towards the surface and further aspirations undertaken through the same insertion site. The aspirated marrow is even richer in stem cells when it is aspirated in small fractions, which reduces the degree of dilution by peripheral blood. All aspirates are pooled in plastic bags containing cell culture medium and anticoagulant solution (citric acid, sodium citrate, dextrose). Pooled aspirates are then filtered to separate cellular aggregates and fat.

The most practical technique is to have two operators aspirating marrow, one on each side of each iliac crest. An assistant places the aspirates in the bag and at the same time rinses the syringes with heparinised medium.

Technique of intra-osseous injection of bone marrow

In fractures. A trocar identical to that used to aspirate the marrow is placed intra-osseously, either at the site of the pseudarthrosis or in the ends of the fracture adjacent to it. The tip of the trocar is positioned by using a brightness amplifier. If in doubt, an opaque medium Hexabrix (Siemens, France) can be injected to check that the marrow has been introduced into the bone. This has been shown to be non-toxic to bone progenitor cells. The injection pressure depends on whether the tip of the trocar is in spongy bone or in the dense fibrous area at the site of the pseudarthrosis. The marrow is injected slowly at a rate of about 20 ml per minute. After injection, the trocar is gradually withdrawn with the core in place, while making small oscillating motions to fill in its path.[32,52]

Since these patients have atrophic nonunion with movement at the fracture site, weight-bearing is not allowed during the first month after bone marrow transplantation in order to avoid mechanical instability interfering with the progression of tissue regeneration and healing. After one month, and only when callus is observed on radiographs, partial weight-bearing is allowed with plaster or external fixation. A period of one month is observed between partial weight-bearing and full weight-bearing. At this time, if the patient has no pain and there is cortical bridging or disappearance of the fracture lines on at least three of the four cortices viewed on the anteroposterior and lateral radiographs, the plaster or external fixation is removed.

In osteonecrosis. The bone marrow is injected into the femoral head using a small trephine (Mazabraud, Collin, France). The instrument is introduced through the greater trochanter as in conventional core decompression.[48] Its position in the femoral head and in the necrotic segment is monitored with fluoroscopy. Since, at the time of treatment, the plain radiographs will show little if any evidence of necrosis, pre-operative MRI scans should be used together with the image intensifier to determine the site of the lesion. The bone marrow is injected through the trephine into the necrotic zone. Although some of the bone-marrow cells may leak through the trephine or into the circulation of the proximal femur, the majority of the bone marrow remains

in the area of osteonecrosis or in the femoral head as has been demonstrated by radionuclide labelling in two patients.[47]

How many cells are necessary for bone repair?

In nonunion. The influence of the number of progenitors on the results of treatment of nonunions of the tibial shaft was evaluated in patients treated with grafting with autologous bone marrow.[53,54] The number of CFU-Fs in bone marrow was determined by assessing the number of nucleated cells and the prevalence of CFU-Fs among them. The number of CFU-Fs which were placed in the graft was determined by the concentration in the original bone marrow aspirate and by centrifugation. The prevalence of connective tissue progenitors in bone marrow in the iliac crests of patients was approximately one per 30 000 nucleated cells.[55] According to the mean nuclear cell count per ml (18 $\times 10^6$ cells), the bone marrow harvested by aspiration from the iliac crest contained on average approximately 600 progenitors per ml in our series.[32,53,54] After preparation of the bone marrow this concentration was increased to approximately 2500 progenitors per ml. The volume of callus as evaluated by a CT scan before and after treatment was on average 3 ml (0 to 5). Each site of nonunion received a mean of 20 ml of bone marrow graft. It is estimated that the volume of bone matrix made by one osteoblast is approximately 5000 cubic micrometres.[56,57] There are approximately 200 million osteoblasts per ml of new bone. Since there are approximately 2500 progenitors per ml of prepared marrow graft, each must have divided a minimum of 16 or 17 times to obtain 1 ml of new bone, assuming that all the bone marrow graft retained the ability to make bone (2500 $\times 2^{16}$ = 163 million osteoblasts) and assuming that 1 ml of bone marrow graft gives 1 ml of new bone.

Another way to evaluate the number of mitoses is to consider that 20 ml of bone marrow graft are necessary to obtain 3 ml of new bone. On this basis the number of mitoses would be 20 instead of 16 or 17. Another consideration is that the differentiation of stem cells is a complex phenomenon.[58] An asymmetrical division of a stem cell[59] can result in one proliferating cell, which will become an osteoblast, and one which will not proliferate. A symmetrical division[60] can result in two proliferative cells, but all the progeny of the initial stem cell will probably not change into the tissue phenotype of interest. Therefore we can only calculate approximately the number of progenitor cells and the number of mitoses which are necessary to produce 1 ml of new bone as seen on a CT scan. Our assessment of the number of mitoses, between 25 and 50, is within the physiological limits found during *in vitro* expansion of similar[60] cells before their senescence.

In osteonecrosis. The influence of the number of progenitors on the treatment of osteonecrosis has been evaluated with MRI.[47] Each femoral head received a mean of 30 ml of bone marrow graft.[47] According to the mean nuclear cell count per ml (29 $\times 10^6$ cells), the bone marrow harvested by

aspiration from the iliac crest contained, on average, approximately 1160 progenitors per ml, which increased the bone marrow concentration to approximately 4900 progenitors per ml. Comparing the MRI scans taken before and after grafting, the volume of the repair was estimated to be 7 cm^3 at one year, 13 cm^3 at two years and 16 cm^3 after four years. However, repair in osteonecrosis is by apposition of new bone on the old matrix with thickened trabeculae and not comparable with the new bone seen as callus after a fracture, so the true volume of new bone formation in osteonecrosis is probably less than indicated by MRI. This has been confirmed by histological examination of the femoral heads of patients treated by hip arthroplasty.[49]

Methods of increasing the number of progenitor cells. Despite the successes that have been obtained using fresh marrow transfer it is frequently impractical to obtain enough bone marrow with the requisite number of osteoprogenitor cells. The reduction of healthy marrow elements which occurs as a consequence of ageing or disease is accompanied by a diminution of the cellular constituents, especially the osteogenic precursors.[61-66] The success of whole marrow grafting is entirely dependent on the transfer of sufficient numbers of these progenitors and so this approach may be least applicable in those situations where it is most needed. In healthy adult marrow osteoprogenitors represent approximately 0.001% of the nucleated cells.[67] Therefore, techniques capable of increasing the volume of progenitor cells are of great clinical benefit. The first to propose the development of an osteogenic bone-marrow preparation were Connolly et al.[68] Two different techniques are usually used to increase the number of cells.

Small aspiration volume to avoid dilution. Because dilution occurs when the volume of aspirate is increased, four 1-ml aspirates provide almost twice the number of progenitors as one 4-ml specimen.[55] The volume should be limited to 2 ml or less in order to maximise the number of progenitors in the graft site.

However, if the primary goal is to obtain as many progenitors as possible with the minimum number of aspirations, and if the concentration of progenitors is not critical, then a volume of 4 ml is preferable. We estimate that this volume will yield 55% or more of the bone-marrow cells aspiratable at a given site. A volume of 10 ml may be appropriate for obtaining bone marrow for a transplantation procedure if success depends primarily on the total number of bone-marrow cells rather than on the concentration of cells in the initial sample.

Concentration. Connolly et al[68] were the first to evaluate the possibility of improving the efficacy of an aspirated bone-marrow graft by concentrating marrow-derived cells. They found a significant increase in bone formation within diffusion chambers implanted *in vivo* in the rabbit. Subsequently, limited clinical experience has been reported using intra-operative centrifuging of marrow for grafting.

We have experience of two techniques: either aspiration, concentration and re-injection during the same operation, which requires that the concentration be achieved in under one hour; or a freezing technique, which allows the marrow to be re-injected at a later date. In this case there is no time limit.

When the marrow is re-injected during the same intervention, it is concentrated in a cell separator (Cobe 2991 (Gambo BCT, Paris, France)). A 5-minute centrifuge at 400 *g* forces the polynuclear cell layer, which is heavier due to the volume of its nuclei, to the periphery, where it can be collected and separated from the remainder. The leucocyte layer is removed at a flow rate of 100 ml/min for 40 to 50 seconds. The lighter, anucleate red cells are found in the centre and are recovered with the plasma. All that remains is the mononuclear layer containing the stem cells. This method reduces 300 ml of bone marrow aspirate to a 'concentrated myeloid' suspension of about 50 ml of stem cells which is poured into a syringe for re-injection.

When the freezing technique is used, the mononuclear cells can be separated on a Ficoll gradient using an IBM-Cobe 2991 red cell washer (Gambo BCT). This technique, which specifically isolates the medullary fraction containing mononuclear cell populations especially rich in stem cells, allows a higher degree of concentration of the aspirate, but takes about two hours, which is too long when re-injection is done during the same intervention as the aspiration. The aspirated marrow is treated on a cell separator prior to freezing so as to reduce the hematocrit and polynuclear cell contamination. Dimethylsulphoxide (DMSO) is used as a cryoprotective agent at a final concentration of 10%. It is diluted in culture medium and added to the cells. DMSO is diluted to 20% in a 4% solution of human albumen. This mixture is progressively added, volume to volume, to the cell suspension at a temperature of 4° to 6°C. When all the solution has been added, the suspension should be frozen as soon as possible because DMSO-induced toxicity in unfrozen cells causes considerable cell loss, even after only a brief contact.

Muschler et al[69,70] have described an alternative and rapid method for the concentration and selection of connective tissue progenitors and other cells from bone marrow using a porous implantable matrix. They showed that the concentration of connective tissue progenitors could be increased in demineralised or mineralised bone matrix having an appropriate pore size and surface area by using the matrix as an affinity column for cells. The connective tissue progenitors can first be attached selectively to the matrix and then transplanted in an appropriate environment into the graft site. They also showed that delivery of connective tissue progenitors at a concentration slightly more than five times that found in a bone marrow aspirate resulted in a significant increase in the rate of union, bone volume and mechanical stiffness. This method provides the opportunity for the design and selection of matrices which are optimised for intra-operative concentration and selection of bone

marrow-derived cells and connective tissue progenitors, and, possibly, other sources of connective tissue progenitors.

Evaluation of the number of progenitor cells in aspirated and re-injected marrow in the operating theatre. The differences between connective tissue progenitors harvested from various individuals are beginning to be understood. They depend on many variables such as age, gender and local and systemic disease. This variability in the osteogenic potential from patient to patient represents a limitation of the technique, and it is difficult for the surgeon to evaluate the number of cells obtained from aspiration. According to our experience[19,32,33,38,42,45-47] with more than 1000 patients, the following recommendations might be useful. The cellularity of bone marrow declines with age and there is also a decrease in the prevalence of connective tissue progenitors with increasing age in women. However, age and gender account for only a small fraction of the variation and connective tissue progenitors can be obtained by bone marrow aspiration in patients of all ages. The total number of progenitors represents the product of the nucleated cells and the prevalence of progenitors in the aspirate. A decline in the number of nucleated cells can be corrected by an increase of the volume aspirated. The number of progenitors can only be determined by a culture but the quantity of medullary nuclear cells can be assessed in the operating theatre.

The quantity of medullary nuclear cells per kg of marrow can be calculated using a formula that takes into account blood dilution. In each ml of aspirate, it was estimated that medullary cells were represented by the difference between the nuclear cell count in the aspirate and that in peripheral blood, which is assessed during general anaesthesia. The number of nuclear cells of presumed medullary origin per kg is expressed as follows:

$$N(10^8/kg) = (V \times NP) - (V - 100) \times NS/P$$

where V, total volume of aspirate in ml, including the harvesting medium; NP, nuclear cell count per ml in the collection bag which leaves the operating theatre, including the harvesting medium; V-100, the exact volume of aspirate, after subtraction of the 100 ml of harvesting medium; NS, the nuclear cell count per ml of peripheral blood drawn during general anaesthesia; and P, patient's weight in kilograms.

Thus, for a total final volume of 300 ml containing 14×10^6 nuclear cells per ml, obtained from a 70-kg adult with a leucocyte count of 4×10^6 per ml as determined under general anaesthesia, it may be estimated that the medullary nuclear cell count is 5×10^7 per kg, for a total of 0.35×10^{10} nuclear cells.

The safety of autologous bone marrow transplantation. Many surgeons now use bone marrow because of its biological value and low risk. One of the authors (PhH) has clinical experience with aspiration of bone marrow in more than 1000 patients. No complications were encountered.

According to Wallden,[71] intra-osseous injection of a therapeutic substance was first described by Josefson in 1934, who administered campolon by direct intrasternal puncture to treat pernicious anaemia. In 1940, Tocantins, O'Neil and Jones[72] suggested the injection of physiological serum into bone marrow in children when the extent of shock was too severe for rapid venous access, although this technique has been superseded. The intra-osseous injection of marrow into the site of nonunion or in osteonecrosis is based on the same principle. Given the permeability of bone tissue to liquid substances,[73] one of the theoretical criticisms of this technique would be the risk of fat embolism during intra-osseous infusion. In a study in dogs, Orlowski et al[74] found fat particles in two pulmonary fields, with one embolus per m^2 at autopsy, although the animals did not have a drop in oxygen saturation or complications during the intra-osseous injection. It is likely that the quantity of fat released is insufficient to produce respiratory distress or to modify arterial pressure in oxygen. In the clinical experience of the authors, no respiratory complications or changes in oxygen saturation have been seen. Yet, considering that the presence of fat particles in pulmonary fields is consistently observed in animals, this technique should not be used in patients with right-left intracardiac shunts due to the risk of embolism to the brain or other vital organs.

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