Development of a per-operative procedure for concentrated bone marrow adjunction in postero-lateral lumbar fusion

Radiological, biological and clinical assessment

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Abstract

Purpose Addition of bone marrow to the bone graft in the postero-lateral lumbar arthrodesis is a widely used technique. Bone marrow brings stem cells and growth factors contained in the platelets, favorable for bone growth. Adjunction of concentrated bone marrow should create better conditions and may increase bone growth.

Methods Simple blind randomized clinical, prospective, monocentric trial was conducted. Fifteen patients underwent lumbar arthrodesis. During surgery, a fraction of the bone marrow harvested was centrifuged. One side received this concentrate with autologous bone and ceramics; the other side received the same graft with unconcentrated bone marrow. A quantitative study, realised with a volume

calculating software on CT-scan images, determined the cortical bone volume in the graft post-operatively and at 3 months. The osteoprogenitor cells, nucleated cells and platelet concentrations were determined.

Results The biological study found an average concentration of six times for the nucleated cells, 3.5 times for the platelets and 2.2 times for the osteoprogenitor cells. The comparison of the mean cortical bone volumes post-operatively and at 3 months was not significantly different.

Conclusions Despite the concentration obtained, there was no increase of bone growth by adding concentrated bone marrow. However, the number of stem cells in bone marrow was low and maybe a stronger concentration is needed to obtain a difference. The 3D reconstruction of the

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graft and the analysis of the graft's volume using a novel software was efficient according to the similarity of the graft's volume post-operatively in all patients.

Keywords Lumbar fusion · Concentrated bone marrow · Per-operative centrifugation · Graft volume quantification

Introduction

Chronic lumbar pain, associated or not with radiculalgia, is a frequent pathology in industrialized countries and medical treatment remains the main therapeutic for the majority of patients. Postero-lateral lumbar arthrodesis is one of the surgical solutions that can be proposed to some patients resistant to the medical treatment. It relies on intertransverse or interarticular bone fusion, bone formation being protected by osteosynthesis.

Autogenous bone harvested from the iliac crests is the standard procedure in bone and joint grafting procedures [1]. But its use exposes to additional risks proper to the harvesting sites such as pain [2], infections, hematomas, wound healing problems [3], fracture and neurological injuries [4]. Among alternative solutions to avoid these morbidities, bone allografts have been tested with poor results compared to autograft, with risks of immunological reaction and potential disease transmission [5].

An actual attractive solution is to use synthetic calcium phosphate ceramics [2]. Macroporous biphasic calcium phosphate ceramics are known to be safe and non-allergenic with good bone-bonding capacity. They contain a combination of two ceramics with different degradation rates: β -tricalcium phosphate (β -TCP) with a rapid degradation rate, and hydroxyapatite (HA) with a slow one. They are used as substitutes for bone grafting in orthopedic, maxillofacial and dental surgery, and have previously shown good results, comparable to autogenous bone graft in spine surgery, especially when used with bone marrow [2, 6, 7]. These osteoconductive ceramics allow propagation of new bone formation on their surface from a pre-existing nearby bone, and need to be associated with an osteoinductive environment. Recombinant human growth factors such as bone morphogenic protein (rh-BMP-2 and rh-BMP-7) have been well documented and are very efficient to stimulate bone growth with ceramics [8] but their approved applications are very restrictive and off label applications have shown a high rate of complications [9]. Furthermore, ceramics and growth factors need osteoprogenitor cells (OPC) to produce bone. Bone marrow, rich in mesenchymal stem cells (MSC) [10], is actually an interesting solution to stimulate bone

fusion [11]. MSC have been shown to differentiate into several types of progenitor cells, through the action of transcription factors that act as “master switches”. RUNX2 transcription factor expression promotes differentiation into OPC which will further differentiate into osteoblasts producing bone matrix [12, 13]. Bone marrow is also rich in platelets which contain many growth factors influencing bone growth and are known to stimulate MSC differentiation and neovascularization in the bone produced [14, 15].

However, over 2 ml of bone marrow aspiration, there is a dilution by peripheral blood, diminishing the concentration in MSC [16]. Thus concentrated bone marrow, concentrating MSC and platelets could be an additional factor to favor bone growth. It has been successfully used in the treatment of leg fractures non-unions [17, 18], which validates its capacity in increasing bone growth in a clinical problem. Platelet concentrates have been found efficient to expand MSC and increase bone formation in vitro and in vivo on calcium phosphate ceramics [19–21]. The current standard concentration procedure is a Ficoll density gradient isolation procedure [22, 23], done in a laboratory which is time consuming. Recently, portable devices which can be used in the operating theater have proved efficient in concentrating bone marrow [24, 25].

The goal of this study was to determine whether bone production in postero-lateral lumbar arthrodesis could be increased using concentrated bone marrow (CBM) with macroporous biphasic calcium phosphate ceramics graft, by comparing it with a standard non concentrated bone marrow (NCBM) with ceramics graft. For this, we used a simple centrifugation technique, which could be done immediately after bone marrow harvesting, during surgery, in the operating theater with a portable device. Cancellous and cortical bone growths were evaluated by a novel radiological method using CT-scan reconstructions. These bone volumes were then correlated to the amount of OPC and platelets injected.

Materials and methods

This was a prospective, monocentric study including 15 patients needing a postero-lateral lumbar arthrodesis of one or two levels, for lumboradiculalgia or low back pain alone, treated medically without success, and with a balanced spine in the frontal view. Each patient was his own control, and received during surgery CBM with graft on one side and NCBM with graft on the other. The sides were chosen by randomization during surgery, just before the grafting. Each patient was informed and signed a consent form realized according to the local ethic committee.

Surgical procedure

We performed standard posterior approaches. Autologous bone harvested during approach (laminar and spinous processes) was minced and quantified in a 60 ml syringe. The autologous bone was mixed with granules of macroporous biphasic calcium phosphate ceramic (Bicalphos[®], Medtronic) to obtain a ratio of one-fourth bone minimum for three-fourth ceramic. This mix was then separated in two equivalent volumes, to graft 5 ml per level on each side. Bone marrow punctures were realized in the posterior iliac crest with a trocar, according to a standardized technique to limit peripheral blood dilution: a small incision was done regarding the posterior iliac crest. A multiperforated trocar was hammered in the posterior iliac crest and pushed 3 cm deep in the bone. Bone marrow was collected with a 60 ml syringe containing 8 ml of anticoagulant (ACD). 5 ml of bone marrow was harvested and the trocar was then extracted 0.5 cm to change the site of suction. Different sites of puncture were realized until the syringe was filled so that 52 ml of BM was harvested. The syringe was permanently agitated, and suction was gentle to limit hemolysis. The bone marrow was then filtered to eliminate micro blood clots and fat and the full-60 ml syringe was then introduced in a centrifuge (Magellan[™], Medtronic) which extracted a 6–8 ml concentrate associating platelet rich plasma and nucleated cells. 3 ml was poured on one part of the mixed bone with ceramics, the other 3 ml was taken to the laboratory for analysis. Bone marrow (NCBM) was harvested again and 3 ml was poured on the same mix of CBM, bone and ceramics. The other half of the bone and ceramic mix received 6 ml of NCBM. 3 ml of NCBM was sent to the laboratory for biological analysis. Posterior instrumentation was realized. These grafts were then

implanted on the intertransverse sites, one on each side after randomization. The wounds were closed over a superficial suction drain which was usually removed on the second postoperative day.

Evaluation procedures

Clinical criteria

Patients record included Oswestry Disability Index, a visual pain scale for radicular pain and for lumbar pain and a SF36 quality of life questionnaire before surgery, at 3, 6, 12 and 24 months after surgery.

Radiological analysis

We performed CT-scans 1 week after surgery and at 3 months. A 3-month evaluation was chosen according to a pilot study (personal data) which found important variability in fusion process on earlier evaluations of the fusion. At 6 months, we feared that the NCBM side fusion could have caught up with the CBM side fusion because evaluation was too late and that the difference in fusion rates could not be visualized any more. Furthermore, several CT-scans at different time points were not realized for ethical reasons to limit exposure of the patients. These CT-scans were analyzed with an innovative software enabling quantification of the graft volume, called Arthrodes[™], developed in collaboration with the Laboratoire de Biomécanique (ENSAM, PARIS). This analysis consisted in surrounding regions of interest, slide by slide and side by side (Fig. 1a). The software reconstructed the volume surrounded and determined volumes inside the graft corresponding to predefined densities (Fig. 1b).

Fig. 1 Arthrodes[™] software. Surrounding the regions of interest on each side after surgery and at 3 months (a). 3D reconstruction of the graft by the software (b)

It thus enabled us to quantify the volume of the graft of cortical bone and cancellous bone. Each side was evaluated 1 week post-operatively and at 3 months, and the cortical bone volume formation between the two CT-scans was determined (the ceramic was included in the cancellous bone volume). We used this variation in cortical bone volumes (which we call cortical bone growth) to evaluate the efficacy of bone fusion of each treatment (CBM and NCBM). The measurement was realized once by one observer, in a blinded manner.

We also performed standard radiographic follow-up of the patients with static and dynamic evaluation at 3, 6, 12 and 24 months. Fusion failure was defined as a second look surgery, implant failure or displacement, clear gap or failure of remodeling on AP views and mobility on dynamic radiographs (>2 mm).

Biological analysis

- Platelet numbers and concentrations were determined by sending 0.5 ml of CBM and NCBM to the biology laboratory.
- Mononucleated cells were isolated by a density gradient centrifugation on Ficoll-hypaque (Biochrom KG, Berlin, Germany). The number of mononucleated cells was counted in the CBM and NCBM.
- 1.5 ml of CBM and NCBM were sent to the INSERM U957 laboratory (Nantes, France) to determine the OPC concentration in CBM and NCBM. For this, 50, 100 and 200 μ l of CBM and NCBM were directly put into culture in 6-well-plates, two wells for each concentration with the medium. These plates were then incubated at 37 °C during 15–21 days (according to the cell growth) in an osteogenic induction medium: α MEM medium (Gibco, France) + 10 % of foetal bovine serum + streptomycin (100 μ g/ml) + glutamine 2 mM + vitamin C 50 μ g/ml. The cells were then marked for phosphatase alkaline activity (Leucocyte Alkaline Phosphatase kit, Sigma-Aldrich, Saint Quentin Fallavier, France). We obtained a visible red-violet marking

for each colony-forming unit which was counted well by well under a microscope. Each CFU was considered as one OPC.

Statistical analysis

All the variables were tested for normality distribution using the Kolmogorov Smirnov test. Standard *t* tests were realized to compare the cortical bone volumes after surgery on each side, to compare the number of OPC injected between CBM and NCBM sides, and to compare cortical bone volumes 1 week after surgery and at 3 months on each side. Linear regression analyses were realized to determine if the number of OPC and the cortical bone volumes after surgery influenced the cortical bone growth. Cortical bone growth between CBM and NCBM sides were analyzed by ANOVA for two factors (bone marrow and patients) without replication. Correlation analyses were realized for cortical bone growths, nucleated cells and platelets concentration, between CBM and NCBM sides.

Results

Clinical and radiological results

The mean age was 46.3 (± 8.5) years old. There were eight female and seven male. Left side received CBM seven times and the right side eight times. None of the patients had complication after surgery. Visual pain scale and ODI were significantly improved at 3 months after surgery compared to before surgery. Radiographic fusion was obtained for all of the patients in both groups at 24 months and no fusion failure was seen.

CT-scan results are shown in Table 1. On the CBM side, the mean cortical bone volume 1 week after surgery was 565.3 mm³. At 3 months, it was 681.9 mm³ (significantly different, $p = 0.009$). Thus the mean cortical bone volume variation was +116.5 mm³. On the NCBM side, the mean cortical bone volume after surgery was 571.4 mm³ (not

Table 1 Radiological and biological analyses results

	NCBM side	CBM side	<i>p</i>
Cortical bone volume after surgery (mm ³)	571.4 (± 379)	565.3 (± 341)	NS
Cortical bone volume after 3 months (mm ³)	669.8 (± 352)	681.9 (± 371)	NS
Cortical bone volume variation (mm ³)	98.4 (± 202)	116.5 (± 166)	NS
Osteoprogenitor cell number injected	1,407 ($\pm 1,987$)	1,701 ($\pm 1,957$)	NS
Concentration of platelets injected (in thousands/ml)	123.5 (± 53)	451.7 (± 343)	0.0005
Concentration of nucleated cells injected (thousands/ml)	7.7 (± 6)	22.4 (± 18.6)	0.002

Means (standard deviation)

NCBM non concentrated bone marrow, CBM concentrated bone marrow

Fig. 2 Correlation between cortical bone growth on CBM side and NCBM side

different statistically from the CBM side). At 3 months, it was 669.8 mm^3 (significantly different from the initial cortical bone volume with $p = 0.04$). The mean cortical bone volume variation was $+98.4 \text{ mm}^3$ which was not significantly different from the CBM side. Linear regression analysis showed there was no correlation between the cortical bone growth and the cortical bone volume in the graft 1 week after surgery. Correlation analysis of the cortical bone growth between CBM and NCBM sides revealed a moderate correlation ($r^2 = 0.365$, $p = 0.017$, Fig. 2).

Biological results (Table 1)

The mean number of nucleated cells obtained in the NCBM was 7.7 ± 6 million/ml and 22.4 ± 18 million/ml in the CBM. We thus obtained a mean concentration rate of 6 (0.64–24.3) for the nucleated cells. The mean number of platelets NCBM was $123.5 \pm 53 \times 10^9/\text{ml}$ and $451 \pm 343 \times 10^9/\text{ml}$ in the CBM. The mean concentration rate for the platelets was thus $\times 3.5$ (0.7–8.78).

The mean number of OPC was 235/ml in the NCBM, and 333/ml in the CBM, with a mean concentration rate of 2.2 (0.93–7.2). Thus, the mean number of OPC injected in the NCBM side was 1,407 ($\pm 1,703$) cells and in the CBM side was 1701 ($\pm 1,556$), the difference was not statistically significant ($p = 0.36$).

Statistical analysis

Linear regression analysis showed no correlation between the cortical bone growth and the number of OPC, the

number of platelets and the number of nucleated cells injected.

Correlation analysis showed no correlation of the number of nucleated cells injected between the CBM and NCBM sides ($r = 0.2$, $p = 0.5$). It showed however a good correlation for the number of platelets injected between the CBM and NCBM sides ($r = 0.69$, $p = 0.0017$) (Fig. 3).

Fig. 3 **a** Correlation between the number of platelets injected in CBM and NCBM. **b** Correlation between the number of nucleated cells injected in CBM and NCBM

Discussion

In this study, we quantified precisely the graft's volume evolution of a postero-lateral arthrodesis graft at 3 months with an imaging technique. It consisted in a volumetric analysis of each component of the graft, cancellous and cortical bone, according to its radiographic density, on CT-scan slides, with a dedicated software. It allowed precise quantification of the volume of cortical bone produced between two follow-up duration (1 week and 3 months), witnessing the bone formation and the quality of arthrodesis. We found that the general graft's volume decreased in the first 3 months (mean -9.1% , no statistical difference between NCBM and CBM sides), mainly because of cancellous bone volume decrease (mean -11.7% , no statistical difference between CBM and NCBM sides). The cortical bone volume increased (mean 33.8%). Results are shown in Table 2. This is considerable advancement in bone formation assessment compared to radiographs analysis which is not reliable because the presence of instrumentation makes it difficult to interpret bony fusion on the standard AP and lateral views, with important inter-observer variations in interpretations, and important false-positive and false-negative rates [26–29]. CT-scan provides better evaluation of lumbar fusion than dynamic radiography and is becoming the preferred method for monitoring patients who have undergone interbody fusion [30]. The software used in this study could also diagnose pseudarthrosis as it rebuilds the graft in 3D with a color density gradient. Slides can be made in the volume, visualizing zones of low density corresponding to the pseudarthrosis. This has been performed once with success and needs further investigation.

We found the same mean cortical bone growth in the graft for the CBM and NCBM sides. We also found important variations in the cortical bone growth between patients, but with the same tendency between the two sides of each patient. The cortical bone growth between the two sides were correlated ($r^2 = 0.365$) and went into the same direction (increase for both sides or decrease for both sides for each patient). For five patients we found a reduction of cortical bone volume after 3 months on both sides. We must see in the long term if this is predictive of bad

outcome concerning pain and pseudarthrosis, but none of the patients showed pain or bad outcome after 2 years follow-up. This also confirms that biphasic ceramics are good materials for grafting in postero-lateral lumbar arthrodesis [31]. We found that there was no correlation between the cortical bone growth and the initial cortical bone volume, and also no correlation between the number of OPC injected and cortical bone growth. However, the number of OPC injected was not significantly different between the sides and it is probable that an important quantity of stem cells must be injected to find a difference, as it has been shown in previous studies [18], which implies an important amount of bone marrow puncture.

We confirmed that a portable device could efficiently concentrate bone marrow with good concentration rates for the platelets and nucleated cells, consistent with the studies of the literature [32]. The OPC concentration rate was 2.2 with no significant difference in the number of OPC injected between the NCBM and the CBM sides. This constitutes the main limitation of our study as it seems that the number of OPC injected is determinant of the amount of bone produced [18]. We found that our centrifugation technique was not reliable as concentration rates were very different from one patient to another. This can be explained either because OPC were damaged or excluded in the concentration process (filtration process applied to bone marrow before concentration, and not for NCBM), either because we had difficulties to identify them. Several studies evaluated these novel methods of bone marrow centrifugation but they mainly focused on the total number of nucleated cells and compare it with the Ficoll concentration process [24]. Only two studies have been found in the literature, which quantify MSC concentrations after bone marrow concentration with a portable centrifugation device. The first one found no difference in the number of CFU after the concentration process compared to Ficoll [25]. Another study found a 14 fold concentration in MSC with a centrifugation device compared to NCBM [33]. It seems that MSC can be well concentrated with portable devices and that our identification process was not optimal, probably because we focused on OPC, and not on MSC. Bone marrow richness before concentration was 76 MSC per million nucleated cells which is consistent with the

Table 2 Grafts and cancellous bone volumes after surgery and at 3 months

	NCBM side	CBM side	<i>p</i>
Graft volume after surgery (cm ³)	7.3 (± 3.1)	7.3 (± 3.2)	NS
Graft volume after 3 months (cm ³)	6.7 (± 2.6)	6.5 (± 2.5)	NS
Cancellous bone volume after surgery (cm ³)	6.8 (± 2.7)	6.7 (± 2.7)	NS
Cancellous bone volume after 3 months (cm ³)	6 (± 2.4)	5.8 (± 2.4)	NS

Means (standard deviation)

NCBM non concentrated bone marrow, CBM concentrated bone marrow

literature [34]. In the present study, it varied a lot between the patients but there was no correlation between the age of the patients and the bone marrow richness.

The platelet concentrates obtained by the centrifugation process of the bone marrow could also have a stimulating effect on bone growth, as it has been showed in preclinical data on ceramics [19]. It had also been shown that platelet concentrates had no effect on instrumented and non-instrumented postero-lateral fusion with autograft alone, without the use of ceramics [35, 36]. We found no benefit in adding CBM, which contain platelet concentrates, to postero-lateral grafting with biphasic ceramic. Thus platelet concentrates do not seem to be a determining factor to stimulate bone growth in instrumented lumbar arthrodesis, whether ceramics are used or not.

Conflict of interest None.

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