# A Prospective Randomized Controlled Trial Comparing Posterolateral Lumbar Fusion With and Without Bone Marrow Concentrate Augmentation in Single-Level Lumbar Spondylolisthesis

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***Background:*** *Bone marrow (BM), which is a good source of stem cells and biological factors, has the potential to enhance bone fusion. Simple centrifugation technique is one of the procedures used to concentrate BM aspirate for increasing number of cells. However, there are limited clinical study for using BM concentrate augmentation in spinal fusion.*
***Objective:*** *This study was designed to examine the spinal fusion enhancement effects of bone marrow (BM) concentrate augmentation on poster lateral lumbar fusion (PLF) with autologous local bone graft in terms of both quality and quantity, as compared with a control procedure without BM concentrate augmentation.*
***Material and Method:*** *Twelve patients with L4-L5 spondylolisthesis scheduled for PLF after decompressive laminectomy and pedicle screw instrumentation were included in this study. This prospective randomized controlled trial was conducted at Siriraj Hospital during the 2009 to 2012 study period. Patients were randomly assigned to two groups. One group underwent PLF with local bone graft with BM concentrate augmentation (BM group) and the other group underwent PLF with local bone graft only (non-BM group). Clinical outcomes were evaluated by the Oswestry Disability Index (ODI) preoperatively and at 3 and 6 months after PLF. Bone fusion quality was evaluated by bony bridging on 3D-CT imaging. Fusion mass volumes were measured on quantitative 3D-CT scans at 1 week and 6 months, postoperatively.*
***Results:*** *Clinical outcome scores did not differ between groups. Six-month postoperative 3D-CT imaging showed complete PLF bridging in 58.3% and 100% of patients in the BM and non-BM groups, respectively. PLF mass volumes were decreased at 6 months by 51.1% in the BM group and by 48.5% in the non-BM group. One patient in the BM group had local inflammation at the BM aspiration site.*
***Conclusion:*** *Bone marrow concentrate augmentation in this small randomized controlled trial failed to demonstrate positive effects on autologous local bone graft in posterolateral lumbar fusion relative to both quality and quantity. The high percentage of incomplete bridging should also be noted and further investigated.*

***Keywords:*** *Bone marrow, Spinal fusion, CT scan, Bone graft, Spondylolisthesis, Posterolateral lumbar fusion*

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Spinal fusion is a surgical procedure that can improve clinical outcomes of treatment in degenerative spondylolisthesis patients. Posterolateral lumbar fusion (PLF) is the one of the fusion procedures available to symptomatic degenerative spondylolisthesis patients who receive decompressive laminectomy[1-3].

For basic bone healing, there are three components required for bone fusion: osteoinduction, osteoconduction, and osteogenic cells[4]. Autologous iliac crest bone graft is the gold standard for spinal fusion because its properties include all three of these required components[5]. Autologous local bone graft is an attractive source for bone grafting, because a local bone graft is made of bone chips or morselized bone obtained from the decompressive laminectomy procedure. However, the biological characteristics of a

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local bone graft, which is mainly composed of cortical bone, are not as favorable as those of an autologous iliac crest bone graft, which mainly contains of cancellous bone, in terms of bone formation mediators and stem cells[6].

Bone marrow (BM), which is a good source of stem cells and biological factors, has the potential to enhance bone fusion, and there are several favorable reports of BM aspiration being mixed with ceramic or local bone graft for PLF[7-9].

Augmentation with a large number of BM cells to increase the number of osteogenic cells, as one of the three required components for bone fusion, is a compelling notion. Simple centrifugation technique is one of the procedures used to concentrate BM aspirate[10]. As the number of BM cells can be increased with a low volume of plasma, augmentation with BM concentrate has the potential to enhance spinal fusion in terms of both quality and quantity of spinal fusion. However, the results reported from previous clinical studies on this topic have been equivocal[11,12].

Accordingly, the present study was designed to examine the spinal fusion enhancement effects of BM concentrate augmentation on PLF with autologous local bone graft in terms of both quality and quantity, as compared with a control procedure without BM concentrate augmentation.

## Material and Method

This single-center, prospective, randomized, controlled trial was conducted during the 2009 to 2012 study period. The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University (No. Si133/2009). Enrolled subjects were single-level L4-L5, low-grade degenerative spondylolisthesis patients who were scheduled to undergo decompressive laminectomy and pedicle screw instrumented PLF procedure. Patients were excluded for any one or more of the following reasons: history of previous lumbar surgery; spinal infection; preexisting medical diseases that could interfere with the fusion process, such as poorly controlled diabetes mellitus, systemic autoimmune inflammatory disease, or end-stage renal disease.

Patients were randomized into two groups (6 patients per group) using computerized randomization software. The intervention group (BM group) comprised of patients who underwent PLF with BM concentrate added to local bone graft. The control group (non-BM group) consistedof patients who underwent PLF with local bone graft only. After being provided verbal and written information regarding the procedure, 12 patients were accepted to participate in this study. Written informed consent was obtained from all 12 patient participants.

### *BM aspiration*

After general anesthesia, patients in the BM group were placed in the prone position and a percutaneous BM aspiration was harvested from the posterior iliac crest. The 10 ml syringe used was prefilled with 0.5 ml of diluted heparin (LEO Pharma A/S, Ballerup, Denmark; 1,000 units in 1 ml) to prevent blood clotting. The plunger of the syringe was drawn back to collect 2 ml of BM. The needle was then advanced 1 cm along the same trajectory, and drawing back of the plunger was repeated until at least 100 ml of BM was obtained. The BM was mixed with heparin in normal saline, yielding a ratio of 20 units of heparin per 1 ml of BM. BM aspirate was collected in a blood bag (T-300; Terumo Corporation, Tokyo, Japan). A 2-ml aliquot of the BM was separated and tested for hematocrit (Hct) and nucleated cells.

### *BM concentration technique*

Aspirated BM in the T-300 blood bag was concentrated using a manual blood bag centrifugation technique. The centrifugation process followed the two-step BM centrifugation protocol previously reported by Sakai et al[10]. The first step involved inverse centrifugation of the blood bag at 1,200 x g for 10 minutes, which separated the red cells into a satellite bag until the interface between the plasma and the red blood cell layer reached a level of 15 mm from the bottom of the bag. The second step involved high-speed centrifugation at 3,870 x g for 7 minutes, by which the plasma was separated by slow transfer into a satellite bag until a minimum amount of plasma remained on top of the buffy coat layer. The bag with the BM concentrate was then sealed using a sterile technique and sent back to the operating theater. Two ml of BM concentrate was evaluated for Hct, nucleated cells, and colony-forming units (CFUs).

### *Surgical techniques*

Surgical procedures were identical in all patients and all procedures were performed by the senior author (AC). Pedicle screws were implanted into the L4 and L5 pedicles bilaterally, and then a decompressive laminectomy was performed to remove the spinal process and lamina in the region of the spinal

canal stenosis. Autologous bone chips extracted during the laminectomy were morselized and collected by meticulously removing the covering soft tissue. After both sides of the transverse processes of L4 and L5 were decorticated, each side of the PLF in the non-BM group was implanted with 5 $cm^3$ of autologous morselized bone chips. In the BM group, each side of the PLF was implanted with a mixture of 5 $cm^3$ of autologous morselized bone chips and 5 mL of BM concentrate.

***Outcome measurements***

Clinical outcomes were evaluated using the Oswestry Low Back Pain Disability Questionnaire (Version 1.0) Thai Version[13] preoperatively, at 3 and 6 months after PLF. The higher the ODI score, the poorer the clinical outcome for back pain.

Radiographic outcomes were evaluated by three-dimensional computed tomography (3D-CT) scans at 1 week and 6 months postoperatively. Distance of each axial image was set at 0.5 mm. Quality of spinal fusion was evaluated by observing the continuity of bony bridging between the L4 and L5 transverse processes. Volume of fusion mass was calculated by 3D volume-rendered reconstruction using AW Volume Share software tool (GE Healthcare, Little Chalfont, United Kingdom) for quantity evaluation of spinal fusion, using the vertebral body as the medial border, upper L4 transverse processes as the upper border, lower L5 transverse processes as the lower border, and most lateral part of the PLF mass as the lateral border. PLF volume was calculated from both sides of the PLF.

***Statistical analysis***

ODI and volume of fusion mass were evaluated by Student's t-test. Quality of fusion and bridging of fusion between L4 and L5 on 3D-CT were evaluated by Pearson's Chi-square test. All statistical analysis were performed by statistical software, SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA). A *p*-value of equal or less than 0.05 was considered statistically significant.

**Results**

The six patients in the BM group included two females and four males with a mean age of 59.5 years (range: 53-72).The six patients in the non-BM group included five females and one male with a mean age of 58.5 years (range: 42-70). There were no smokers in either group.

In the BM group, the volume of red blood cells in the BM increased from 30.5% (range: 22-38%) to 66.4% (range: 52.5-76.4%) after the centrifugation process. The number of white blood cells in the BM increased from $18.1x10^3$cells/μL (range: $11x10^3$-$35.6x10^3$) to $181.3x10^3$cells/μL (range: $47.5x10^3$-$412x10^3$). The volume of the BM decreased from 162 mL (range: 124-217 ml) to 22.9 mL (range: 12- 42 ml). The colony-forming unit (CFU) results for the BM aspiration were more than 50 CFUs in all patients.

All patients had complete follow-up according to the study protocol. ODI scores did not differ significantly between the two groups preoperatively or at 3 and 6 months postoperatively (Fig. 1). Overall ODI scores in both groups were decreased, reflecting improved clinical outcomes after the surgery, but there were no significant differences in clinical outcomes between groups ($p>0.05$).

In the BM group, incomplete bridging of fusion was observed in three patients (two patients on both sides and one patient on one side). All 6 patients in the non-BM group achieved complete bridging bone fusion on both the left and right sides at 6 months postoperatively; a finding that differed significantly from that of the BM group ($p = 0.01$).

Volume of fusion mass from 3D-CT measurements did not differ significantly between groups. Volume of fusion bone graft at 6 months after surgery decreased in both groups by around half from the immediate postoperative bone volume (Fig. 2).

A complication was observed in one patient in the BM group who had local inflammation at the aspiration wound for 1 week, but had no fever. This patient received an extended course of antibiotics for 1 week and the complication was resolved.

**Discussion**

The results of using autologous local bone graft augmented with BM concentrate with the aim of improving the characteristics of autologous local bone graft did not show enhancement of bone fusion in terms of either quantity or quality in this study. In a comparison between groups, quality of fusion mass in the BM group was lower than that of the non-BM group; however, there was no difference between groups for fusion mass quantity. Resorption rate of the bone graft was nearly 50% in both groups at 6 months after PLF.

Although augmentation with BM aspirateto enhance spinal fusion has been reported in previous studies, most of those studies used non-concentrated BM aspirate[7,8,14]. A few previous prospective studies

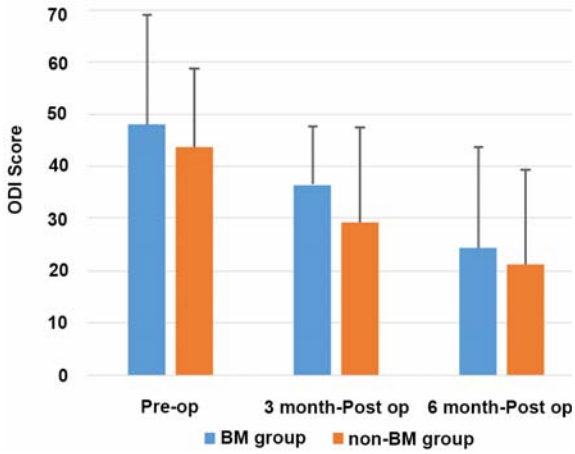


**Fig. 1** Comparison of the ODI scores between the BM group and the non-BM group.

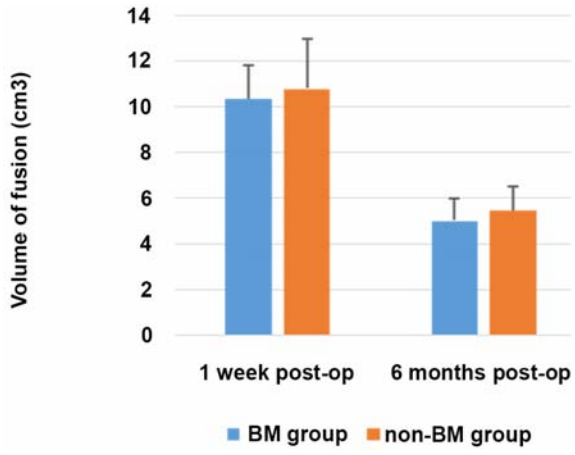


**Fig. 2** Comparisons of the volume of the fusion mass between the BM group and the non-BM group.

investigated concentrated BM aspiration in spinal fusion, but the results from those studies were equivocal. In a study by Gan et al[11], 41 patients received instrumented posterior fusion with concentrated BM aspiration combined with a ceramic bone substitute and two patients in that study had nonunion. In a study by Taghavi et al[12], 62 patients who received instrumented PLF were divided into the following three groups: auto graft, BMP2 and concentrated BM aspiration with allograft. The lowest fusion success rate was observed in the concentrated BM aspiration with allograft group (77.8%), while the other 2 groups had fusion success rates of 100%. Moreover, the time to solid fusion was longest in the concentrated BM aspiration with allograft group. A recent study by Johnson[15] compared an autologous iliac crest bone graft with an allograft combined with concentrated BM aspirate in a side-by-side human model. The results between groups were equivalent.

There are some potential explanations why we observeda poorer quality of fusion in the patients who received concentrated BM augmentation in this study. First, the heparin that was added to prevent clotting of the BM may have disturbed the bone healing processes, which involve the need to form a clot at the fusion site. There is a previous report that describes interference with clotting in an animal model that resulted in delayed fusion[16]. Heparin also interferes with the differentiation of osteoblasts and promotes osteoclastic bone resorption, which causes significantly more bone loss by both decreasing the rate of bone formation and increasing the rate of bone resorption[17-19]. Citrate-based anticoagulants are alternative coagulants that may be used in the BM concentration procedure, but there is limited evidence in spinal fusion models. Adding substances like thrombin or calcium chloride to reverse the anticoagulant effect is another option. In the study by Johnson[15], a bone graft "log" made from an allograft, concentrated BM aspiration, recombinant thrombin, and calcium chloride showed good results.

Second, the simple centrifugation technique used in this study achieved a mixed-cell population of BM cells tocombine withthe local bone graft and this centrifugation process could not strictly control the number of purified osteogenic progenitor cells from the BM aspiration[10]. The number and quality of osteoprogenitor cells from the BM aspiration can vary and are specific to individual patients, based on factors such as age, menopausal status, and diseases[20,21].

Third, a large number of cells in the transplanted concentrated BM aspirate may not have survived owing to a lack of nutrition, low oxygenation, or waste product toxicities-resembling problems reported in the use of purified cells in transplantation for other diseases[20]. These factors may have adversely affected the quality of fusion.

Radiographic outcomes in the present study did not demonstrate benefit of augmentation with concentrated BM aspirate. There was a high percentage of incomplete bridging of the fusion mass in the BM group, although there was no difference between groups regarding volume of fusion mass at 6 months after PLF. Even though the clinical outcomes between groups at 6 months did not differ significantly, long-term negative clinical outcomes should be of concern. In a previous study, instrumentation in PLF was shown

to produce immediate stability during the early postoperative period, implying that a short follow-up period may not reveal any identifiable differences in clinical outcomes[22]. Complete, high quality fusion may be an important factor for long-term clinical outcomes, as nonunion in spinal fusion can cause implant loosening or breakage, which results in a need for revision surgery[23].

BM aspiration is a safe procedure. However, some complications have been reported, including pain, infection, fracture and sciatic nerve injury[24]. In the present study, one patient developed local inflammation at the BM aspiration wound. The potential for complications should be considered, even though most related complications are minor.

Although this study had a small number of patients, an uncontrolled number of cells, a lack of biological substance transfusion data, and a short-term follow-up period, the results of this study do not support the efficacy of autologous local bone graft augmented with concentrated BM aspirate in instrumented PLF patients, based on precise measurements using fine-cut CT imaging.

## Conclusion

Bone marrow concentrate augmentation in this small randomized controlled trial failed to demonstrate positive effects on autologous local bone graft in poster lateral lumbar fusion relative to both quality and quantity. The high percentage of incomplete bridging should also be noted and further investigated.

## What is already known this topic?

Poster lateral lumbar fusion and decompressive laminectomy is a good procedure to treat symptomatic degenerative spondylolisthesis patients. Simple centrifugation can increase the bone marrow number of cells.

## What this study adds?

Bone marrow concentrate augmentation failed to demonstrate positive effects on the posterolateral lumbar fusion relative to both quality and quantity.


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## Potential conflicts of interest

None.


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# การศึกษาเปรียบเทียบการเชื่อมกระดูกสันหลังในผู้ป่วยที่มีกระดูกสันหลังเคลื่อนระดับเอวโดยระหว่างการใช้และไม่ใช้ไขกระดูกเข้มข้น

อารีศักดิ์ โชติวิจิตร, มนต์ชัย เรืองชัยนิคม, ตรงธรรม ทองดี, อดิศักดิ์ วงศ์ขจรศิลป์, ปาริชาติ เพิ่มพิกุล, เอกพจน์ ก่อวุฒิกุลรังษี

**ภูมิหลัง:** ไขกระดูกมีส่วนประกอบทั้งเซลล์และสารที่ช่วยในการติดของกระดูก การใช้ไขกระดูกเข้มข้นที่มีปริมาณเซลล์มากขึ้นมีความน่าสนใจในการนำมาใช้เพื่อช่วยในการเชื่อมกระดูกสันหลังสำหรับการรักษาผู้ป่วยที่มีการเคลื่อนกระดูกสันหลังระดับเอว แต่ข้อมูลสำหรับการนำมาใช้ในการรักษายังมีจำกัด

**วัตถุประสงค์:** ศึกษาเปรียบเทียบผลการเชื่อมกระดูกสันหลังในผู้ป่วยที่มีกระดูกสันหลังเคลื่อนระดับเอวโดยระหว่างการใช้และไม่ใช้ไขกระดูกเข้มข้นทั้งในแง่ปริมาณและคุณภาพ

**วัสดุและวิธีการ:** การทดลองทางคลินิกแบบสุ่มและมีกลุ่มควบคุมโดยแบ่งผู้ป่วยที่มีกระดูกสันหลังเคลื่อนระดับเอวข้อที่ 4 และ 5 ที่ได้รับการผ่าตัดลดการกดทับเส้นประสาทและผ่าตัดเชื่อมกระดูกสันหลังร่วมกับการใช้โลหะดามกระดูก โดยการทดลองแบ่งผู้ป่วยเป็น 2 กลุ่ม กลุ่มทดลองมีผู้ป่วย 6 คนที่ได้รับการใช้ไขกระดูกเข้มข้น กลุ่มควบคุมมีผู้ป่วย 6 คน ซึ่งไม่ได้ใช้ไขกระดูกเข้มข้นในการรักษา ดำเนินการทดลองตั้งแต่ปี พ.ศ. 2552-2555 เก็บข้อมูลผู้ป่วยเปรียบเทียบทั้งอาการผู้ป่วยโดยใช้คะแนน Oswestry disability index (ODI) และการประเมินการติดของกระดูกทั้งปริมาณและคุณภาพด้วยการถ่ายภาพรังสีส่วนตัดอาศัยคอมพิวเตอร์

**ผลการศึกษา:** จากการประเมินอาการของผู้ป่วยด้วยคะแนน ODI ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างการทดลองสองกลุ่ม ส่วนการติดของกระดูกในเดือนที่ 6 กลุ่มทดลอง (กลุ่ม BM) มีอัตราการติดกันของกระดูกร้อยละ 58.3 กลุ่มควบคุม (กลุ่ม non-BM) มีอัตราการติดกันของกระดูกร้อยละ 100 โดยที่ขนาดของกระดูกที่เชื่อมไม่แตกต่างกันจากการวัดในภาพรังสีส่วนตัดอาศัยคอมพิวเตอร์ และพบว่าขนาดของกระดูกที่เชื่อมทั้งสองกลุ่มมีปริมาตรลดลงที่ 6 เดือนหลังผ่าตัด (ร้อยละ 51.1 ในกลุ่มทดลองและร้อยละ 48.5 ในกลุ่มควบคุม) ในกลุ่มทดลองมีผู้ป่วยหนึ่งคนเกิดแผลอักเสบบริเวณที่ได้รับการเจาะไขกระดูก

**สรุป:** ในการทดลองนี้การใช้ไขกระดูกเข้มข้นไม่สามารถแสดงผลบวกต่อการเชื่อมกระดูกสันหลังระดับเอวสำหรับการผ่าตัดรักษาผู้ป่วยที่มีกระดูกสันหลังเคลื่อนได้ นอกจากนั้นยังต้องเฝ้าระวังการติดของกระดูกที่เชื่อมถ้ามีการใช้ไขกระดูกเข้มข้นร่วมด้วย