***Original article***

# Clinical performance of a collagen-based hydroxyapatite bone graft substitute in procedures of spinal arthrodesis

A. BARBANERA*, M. VITALI**, A. NATALONI**, V. CANELLA*

** Division of Neurosurgery, "SS. Antonio and Biagio and Cesare Arrigo" Hospital, Alessandria, Italy*
*** Clinical Department, Fin-Ceramica, Faenza, Italy*

***SUMMARY: Aims.*** *Spinal fusion is a common procedure used for surgical treatment of spinal deformity. In recent years, many bone graft substitutes have been developed to provide good arthrodesis when the available autologous bone harvested from the patient is not enough. Besides, bioactive synthetics have undergone changes to stimulate a beneficial response within the bone, which are based on chemical reactions allowing cellular turnover and the enhancement of new bone formation. In this context, a collagen-based hydroxyapatite bone graft substitute enriched in magnesium has shown promising results in achieving fusion for the treatment of adult scoliosis. Aim of the present clinical study was to evaluate the performance and safety of a collagen-based hydroxyapatite bone graft substitute enriched in Magnesium, to stimulate bony fusion in patients undergoing posterolateral spinal fusion.*
***Materials and Methods.*** *Twenty patients were consecutively enrolled and prospectively evaluated. Patients underwent instrumented posterolateral spinal fusion using a biomimetic hydroxyapatite composite scaffold. Radiological evaluations were performed at 12 months follow-up to evaluate fusion.*
***Results.*** *The percentage of bony fusion recorded was of 95% at twelve months follow-up. No intra-operative or post-operative adverse events were recorded.*
***Conclusions.*** *The present study provides clinical evidences of the fusion properties of a collagen-based HA scaffold, enriched in magnesium, for posterolateral spinal fusion. The safety profile and the osteointegrative properties makes the device a valid alternative to local autograft bone.*

***KEY WORDS:*** *Bioinorganic ions, Bone graft substitutes, Mg-enriched hydroxyapatite, Posterolateral fusion.*

## INTRODUCTION

Vertebral arthrodesis is one of the most common surgical procedures for the treatment of deformities, trauma and degenerative pathologies affecting the spinal column[17]. Thanks to the use of bone grafts and instruments (i.e. metal rods and screws), the procedure allows to create fusion between two or more adjacent vertebrae and to stabilize the vertebral column. The biological processes involved in bone regeneration require three critical elements: an osteogenic potential that is capable of directly providing cells to the newly forming bone, osteoinductive factors able to cause osteoblastic differentiation of osteoprogenitor stem cells, and the presence of an osteoconductive scaffold that facilitates neo-

Correspondence: Dr.ssa Valentina Canella, Clinical Department, Fin-Ceramica, via Ravegnana 186, 48018 Faenza (RA), Italy, ph. +39-(0)546 607355, e-mail: valentina.canella@finceramica.it
Progress in Neurosciences 2020; 5 (1-4): 3-9. *ISSN: 2240-5127.*

***LIST OF ACRONYMS AND ABBREVIATIONS:*** **BMPs** = Bone Morphogenetic Proteins; **DBM** = Demineralized Bone Matrix; **HA** = HydroxyApatite; **Mg-HA** = Magnesium doped-HydroxyApatite; **TCP** = TriCalcium Phosphate.

vascularization and supports the bone ingrowth[15]. An ideal bone graft should possess all these features, together with excellent biological compatibility and biological safety[4].

To date, autologous bone, either local or harvested from the iliac crest, is still considered the "gold standard", thanks to its osteoinductive, osteoconductive and osteogenic properties. However, contraindications and drawbacks have been frequently reported, such as limited quantity and donor site pain[17]. In order to overcome these limitations, many alternatives have been developed during decades and are currently available on the market, among which growth factors (BMPs), allogeneic or heterologous bone, DBM and synthetic ceramic materials[1,10]. However, although being extensively investigated[1,2,10,17], clinical data concerning these alternative materials are still scarce in terms of quantity and quality, type of studies, evaluations performed and conclusions reached[10,17]. As such, an understanding of the precise biological mechanism of each bone substitute is necessary for achieving successful results.

Synthetic ceramic-based bone graft substitutes, such as beta-TCP and HA, have been developed as osteoconductive scaffolds able to fill defects, support bone remodelling and regeneration, thanks to their chemico-physical features and properties which are very similar (up to 70%) to the human mineral bone component[2,10].

In the last years, moreover, the incorporation of bioinorganic ions such as silicon, magnesium, strontium, zinc and copper, has been extensively investigated[20], being actively involved in ion channel processes and cellular signalling recruiting, specifically, osteoblasts and osteoclasts for regenerative processes of new bone formation[20]. The therapeutic use of bioinorganic ions, such as magnesium, doped into various materials, including hydroxyapatite, tricalcium phosphate and collagen, showed osteoblastic cellular attachment, proliferation and newly bone formation in in vivo experiments[13] and clinical applications[7]. Additionally, the incorporation of these ions in synthetic bone grafts like hydroxyapatite confers low cost, longer shelf life and lower risk as compared to other bone graft alternatives[20].

## ☐ AIMS

In the present clinical study, we investigated the use of a biomimetic HA scaffold, enriched in magnesium and type I collagen of equine origin. Hydroxyapatite represents the majority of the human mineral bone component (65% weight) while type I collagen is a fibrous protein representing the most important structural component of the extracellular matrix of many human tissues. The presence of type I collagen allows the scaffold chemico-physical biomimetic properties, ensuring great stability on site. The three-dimensional and flexible composite scaffold has already been used in vertebral arthrodesis procedures involving long tracts of the column spine, showing good results and a safety profile. The study was set up as a spontaneous, prospective, observational, post-marketing clinical study, with 12 months follow-up.

## ☐ MATERIALS AND METHODS

In this prospective observational data collection, we consecutively screened and enrolled patients who had indications for single or multi-level instrumented posterolateral fusion due to symptomatic degenerative disc disease. Primary endpoints were bone regeneration and fusion, intended as the presence of continuous trabecular bone bridge verified by diagnostic imaging (X-ray) and assessed by the Brantigan score; the safety of the medical device, through the incidence of any adverse events, complications, unexpected reactions, accidents.

The Medical Ethic Committee of the Ospedale di Stato della Repubblica di San Marino (Italy) was informed (in conformity to the 1975 *Declaration of Helsinkj*).

The following criteria for inclusion were considered: skeletally mature subjects, at least 18 years of age at the time of surgery, affected by symptomatic degenerative disc disease (i.e. disc herniation, lumbar stenosis, spondylolysis with spondylolisthesis); with indication for posterolateral fusion in the L1-S1 lumbosacral tract.

Exclusion criteria were: alcohol or drugs abuse; active or systemic local infections, drug therapy resulting in impaired bone regeneration (use of cortico-

| Brantingan and Steefe classification system | | |
|---|---|---|
| ***Fusion score*** | | |
| **GRADE 1** | UNFUSED | Obvious radiographic pseudarthrosis, based on collapse of the construct, loss of disk height, vertebral slip, broken screws, cage displacement, or resorption of the bone graft |
| **GRADE 2** | PROBABLY UNFUSED | Probable radiographic pseudarthrosis, based on significant resorption of the bone graft, or a major radiolucency or visible gaps in the fusion area |
| **GRADE 3** | UNCERTAIN | Bone graft is visible in the fusion area approximately at the same density originally achieved intraoperatively. A small radiolucency or gap may be visible involving a portion of the fusion area, but at least half of the graft area showing no radiolucency between the graft bone and vertebral bone |
| **GRADE 4** | POSSIBLY FUSED | Bone bridges the entire fusion area with at least the density originally achieved intraoperatively. No radiolucency between the donor bone and vertebral bone should be present |
| **GRADE 5** | FUSED | The bone in the fusion are is radiographically denser and more mature, as compared to the intraoperative phase. No radiolucency detectable between the bone graft and the host bone |

**Table 1.** The Brantingan and Steefe classification system.

steroids, chemotherapeutic drugs, etc.); active malignancy, metabolic or haematic disorders; pregnancy; inflammatory or auto-immune pathologies, hypercalcemia, insulin-dependent diabetic conditions, thyroid function impairment, allergy to equine collagen and calcium phosphate salts.

At pre-operatory, patients' demographic data and clinical history were recorded. X-ray and CT scan were undertaken. The number of vertebral levels fused during surgery was recorded. Patients were followed post-surgery until discharge. X-ray and CT scans were undertaken just after surgery and at 12 months follow-up. Follow-up visits were conducted at 12 months. Any intra-op or post-op adverse event was recorded.

■ **Biomaterial.** The bone graft substitute employed in this study (RegenOss, provided by Fin-Ceramica Faenza S.p.A., Faenza, Italy) is a commercially available, porous, three-dimensional composite bone graft substitute made of type I collagen fibres (of equine origin) in which nano-sized (10-20 nm) crystals of biomimetic Mg-HA are nucleated at a 40-60% ratio. The composite device is manufactured to reproduce the anatomical structure of the bone compartment in the biological processes of neo-ossification. The device is biocompatible. While the bone tissue regeneration proceeds, the device undergoes resorption.

■ **Surgical technique.** All the patients underwent decompression and spinal stabilization using instrumented fixation supports (pedicle screws/rods/cage) in one or more spinal levels between L1 and S1. All the surgical procedures were performed by the same senior surgeon by using the standard open posterior approach to the lumbar sacral spine. Patients underwent intravenous antibiotic treatment 30 minutes before surgery (cefazolin 2 g total amount). Pedicle titanium screws (Expedium system; DePuy Synthes) were used. A bleeding bone fusion bed was obtained through decortication of the posterolateral area from the transverse processes throughout the posterior aspect of the facet joints. The bone graft substitute RegenOss was located by one side of the defect, while autologous bone was placed by the opposite side of the spinal tract to be fused. The wound was sutured in three layers over two suction drainage tubes. Patients were intravenously treated with prophylactic antibiotic therapy immediately after surgery (cefazolin 2 g, total amount) and mobilized for 2 days after surgery. Follow-up visits, including the recording of clinical parameters and radiological analysis, were conducted at 12 months follow-up.

■ **Results assessment.** The degree of fusion was determined using plain radiography and evaluated through the Brantingan and Steefe classification system[3] (Table 1), modified as follows: fusion was considered to be successful with radiographic evidences of mature bony trabecular bridging into the fusion area, no signs of pseudoarthrosis, no signs of interspaces between the bone graft and the host bone. Pseudoarthrosis was defined in case of implant

mechanical collapse, reduction of the intervertebral space, vertebral body sliding, breaking of the screws, or resorption of the bone graft. Pseudoarthrosis (i.e. the lack of fusion) was considered as adverse event. The safety of the medical device was evaluated by recording adverse events or any complication occurring to the patients.

■ **Statistical analysis.** Values are presented as number, mean or percentage, as appropriate. Given the small sample size, no statistical analysis was performed.

## □ RESULTS

Twenty patients satisfied the inclusion criteria and were enrolled in the study protocol between 2015 and 2016. There were eleven female (55%) and nine male (45%), mean age 56.4 years (age range: 38-71). All the patients underwent spinal surgery because of degenerative pathologies. Fifteen patients (75%) underwent one level spinal fusion (ten female and five male), whiles the remaining (25%) underwent two fusion levels (one female and four male). All the patients underwent radiographic control at 12 months follow-up.

At follow-up, fusion was assessed by X-ray and evaluated according to the criteria described by Brantigan. Nineteen out of 20 patients (95%) showed a Brantingan score equivalent to Grade 5. Only one patient showed an uncertain radiological outcome (Grade 3). No major complications related to the surgical procedure were recorded in both the treatment groups.

Figure 1 and Figure 2 report due examples of radiographic fusion reached at 12 months follow-up.

## □ DISCUSSION

Currently, autologous bone graft is still considered the "gold" standard material for achieving good arthrodesis. However, the relatively limited quantity of local bone makes the need to identify other bone graft sources[20]. Harvesting autograft bone from the iliac crest is a possible solution, and a standard procedure as well, but complications associated with this technique are well known: donor site morbidity, postoperative pain, hematoma, infections and increased blood loss, which may occur in 25%-30% of patients, thus limiting its use[19]. Other alternatives are represented by allograft bone harvested from a cadaveric donor, which is often associated with potential risk of disease transmission, bacterial contamination or host-related reactions[5], or DBM. a kind of highly processed allograft derivative with at least 40% of the mineral component removed by chemical treatments, which negatively impact on the structural integrity and mechanical properties of the material[11,20].

The most common and safe alternative is therefore represented, since years, by synthetic biomaterials such as ceramics.

The use of ceramic-based BGSs in spinal applications has been widely investigated during recent years. In a level I study, Korovessis et al.[12] reported progressive fusion at 12 months follow-up in 60 patients undergoing posterolateral fusion. Yoo et al.[21] showed no statistical significant difference between HA and autologous fusion rate in two different patient groups. The fusion rate in both groups was of about 90% at 2 years follow-up. Nickoli and colleagues[18] reviewed 30 clinical studies using ceramic-based materials as bone graft extenders in the lumbar spine. In 10 studies, involving more than 450 patients, the use of ceramics plus local autograft evidenced a fusion rate of around 90%. Lee and coworkers[14] reported no difference between the patient group treated with HA (87%) and the control group treated with ICBG (89%) in terms of fusion rates. Korovessis et al[12] concluded that HA together with the use of instrumentation and autologous bone provides good performance and a solid dorsal fusion within the expected time. Mashhadinezhad et al[16] evaluated the degree of fusion after applying HA inserted into cages for interbody fusion. The authors reported no difference between fusion rates achieved with HA compared with ICGB at 12-month follow-up, showing that application of HA granules, even inserted in cages, proved to be an effective treatment also for interbody fusion applications. All of these data again confirm the safety and effectiveness of HA-based bone grafts in different spinal applications. In this context, the HA-based device RegenOss, enriched in magnesium and type I collagen, has shown fusion and regenerative properties, as expected. Subjects implanted with RegenOss confirm an improvement of bony fusion at 12 months follow-up and a safety profile, as previously reported by others. Clinical data on the performance of RegenOss in spinal arthrodesis are well known since years. In October 2013, results from a prospective, observational study ("Lumbar posterolateral fusion using a biomimetic and bioinspired bone graft: a prospective

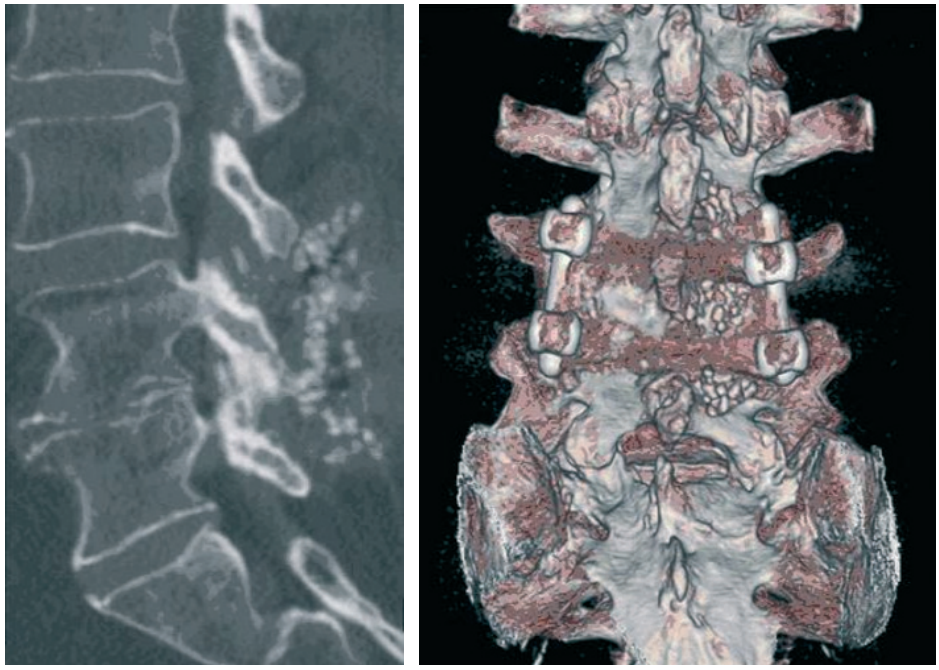

**Figure 1.** Male, 52 years old, 1 lumbar level treated between L3 and L4. Radiographic and CT scan images showing complete fusion (grade 5 Brantingan scale) at 12 months follow-up.

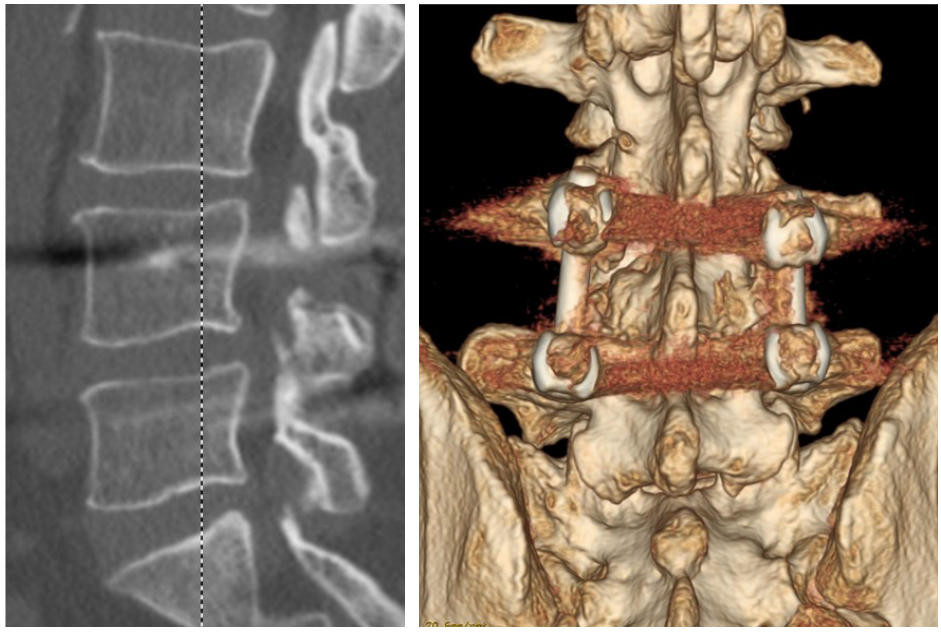

**Figure 2.** Female, 71 years old, 1 lumbar level treated between L2 and L3. Radiographic and CT scan images showing complete fusion (grade 5 Brantingan scale) at 12 months follow-up.

observational, non-randomized, single arm clinical study") were presented at the "Materials in Medicine Conference" held in Faenza (Italy). The study showed the regenerative properties of the bone graft substitute RegenOss already at six months follow-up, with complete new bone formation and bony fusion was observed in 80% of the cases. Recently, Giorgi and colleagues[7] published the results of a clinical study on the use of RegenOss for long tracts of posterolateral fusion (i.e. scoliosis), showing radiographic evidences of bony fusion in the majority of patients (more than 70%) and reduction of pain at 12 months follow-up, which was improved (95% fusion) at subsequent follow-up periods (36-months). The device showed no complications or side effects even when employed for long tracts spinal tracts. Additionally, in vitro experiments and preclinical tests[6,9] well-documented the ability of the scaffold RegenOss

to support proliferation and differentiation of mesenchymal stem cell, providing a biochemical environment able to promote bone regeneration. A case report by Grigolo and colleagues[8] documented the regenerative properties of the device, together with fast degradation and quick resorption. In this case specifically, the rupture of a metallic bar brought to spinal revision surgery at 14 months follow-up, allowing to document the degree of bone fusion achieved by RegenOss. Histological and immunohistochemical analyses performed on a biopsy sample showed the complete resorption of the device, together with good quality of new bone formation.

## ☐ CONCLUSIONS

This study has shown that RegenOss, a biomimetic three-dimensional scaffold made of HA enriched in magnesium, provides arthrodesis in posterolateral fusion. The use of the 3D scaffold provides new bone formation, representing a valid solution for the achievement of spinal fusion.

## ☐ REFERENCES


1. Abdullah KG, Steinmetz MP, Benzel EC, Mroz TE. The state of lumbar fusion extenders. Spine 2011; 36 (20): E1328-1334.
2. Alsaleh KAM, Tougas CA, Roffey DM, Wai EK. Osteoconductive bone graft extenders in posterolateral thoracolumbar spinal fusion: a systematic review. Spine 2012; 37 (16): E993-1000.
3. Brantigan JW, Steffee AD. A carbon fiber implant to aid interbody lumbar fusion. Two-year clinical results in the first 26 patients. Spine 1993; 18 (14): 2106-2107.
4. Campana V, Milano G, Pagano E, Barba M, Cicione C, Salonna G et al. Bone substitutes in orthopaedic surgery: from basic science to clinical practice. J Mater Sci Mater Med 2014; 25 (10): 2445-2461.
5. Delloye C, Cornu O, Druez V, Barbier O. Bone allografts: What they can offer and what they cannot. J Bone Joint Surg Br 2007; 89 (5): 574-579.
6. Ding M, Koroma KE, Sorensen JR, Sandri M, Tampieri A, Jespersen SM et al. Collagen-hydroxyapatite composite substitute and bone marrow nuclear cells on posterolateral spine fusion in sheep. J Biomater Appl 2019; 34 (3): 365-374.
7. Giorgi P, Capitani D, Sprio S, Sandri M, Tampieri A, Canella V et al. A new bioinspired collagen-hydroxyapatite bone graft substitute in adult scoliosis surgery: results at 3-year follow-up. J Appl Biomater Funct Mater 2017; 15 (3): e262-e270.
8. Grigolo B, Dolzani P, Giannetti C, Tenucci M, Calvosa G. Use of a fully-resorbable, biomimetic composite hydroxyapatite as bone graft substitute for posterolateral spine fusion: A case report. Int J Clin Exp Med 2016; 9 (11): 22458-22462.
9. Grigolo B, Fiorini M, Manferdini C, Cavallo C, Gabusi E, Zini N et al. Chemical-physical properties and in vitro cell culturing of a novel biphasic bio-mimetic scaffold for osteo-chondral tissue regeneration. J Biol Regul Homeost Agents 2011; 25 (2 Suppl): 3-13.
10. Hsu WK, Nickoli MS, Wang JC, Lieberman JR, An HS, Yoon ST et al. Improving the clinical evidence of bone graft substitute technology in lumbar spine surgery. Glob Spine J 2012; 2 (4): 239-248.
11. Kadam A, Millhouse PW, Kepler CK, Radcliff KE, Fehlings MG, Janssen ME et al. Bone substitutes and expanders in Spine Surgery: A review of their fusion efficacies. Int J Spine Surg 2016; 10: 33.
12. Korovessis P, Koureas G, Zacharatos S, Papazisis Z, Lambiris E. Correlative radiological, self-assessment and clinical analysis of evolution in instrumented dorsal and lateral fusion for degenerative lumbar spine disease. Autograft versus coralline hydroxyapatite. Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc 2005; 14 (7): 630-638.
13. Landi E, Logroscino G, Proietti L, Tampieri A, Sandri M, Sprio S. Biomimetic Mg-substituted hydroxyapatite: from synthesis to in vivo behaviour. J Mater Sci Mater Med 2008; 19 (1): 239-247.
14. Lee JH, Hwang C-J, Song B-W, Koo K-H, Chang B-S, Lee C-K. A prospective consecutive study of instrumented posterolateral lumbar fusion using synthetic hydroxyapatite (Bongros-HA) as a bone graft extender. J Biomed Mater Res A 2009; 90 (3): 804-810.
15. Ludwig SC, Kowalski JM, Boden SD. Osteoinductive bone graft substitutes. Eur Spine J 2000; 9 (Suppl 1): S119-S125.
16. Mashhadinezhad H, Samini F, Zare R. Comparison of outcomes and safety of using hydroxyapatite granules as a substitute for autograft in cervical cages for anterior cervical discectomy and interbody fusion. Arch Bone Jt Surg 2014; 2 (1): 37-42.
17. Miyazaki M, Tsumura H, Wang JC, Alanay A. An update on bone substitutes for spinal fusion. Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc 2009; 18 (6): 783-799.
18. Nickoli MS, Hsu WK. Ceramic-based bone grafts as a bone grafts extender for lumbar spine arthrodesis: a systematic review. Glob Spine J 2014; 4 (3): 211-216.
19. Park JJ, Hershman SH, Kim YH. Updates in the use of bone grafts in the lumbar spine. Bull Hosp Jt Dis 2013; 71 (1): 39-48.

20. Wang W, Yeung KWK. Bone grafts and biomaterials substitutes for bone defect repair: A review. Bioact Mater 2017; 2 (4): 224-247.
21. Yoo J-S, Min S-H, Yoon S-H. Fusion rate according to mixture ratio and volumes of bone graft in minimally invasive transforaminal lumbar interbody fusion: minimum 2-year follow-up. Eur J Orthop Surg Traumatol Orthop Traumatol 2015; 25 (Suppl 1): 183-189.