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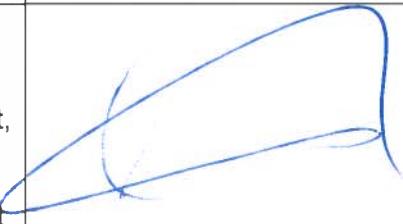
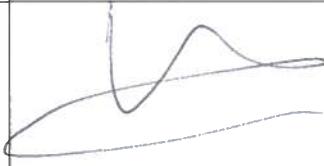
**'A Randomized Intra-Patient Controlled Trial
of MagnetOs™ Granules vs. Autograft
in Instrumented Posterolateral Spinal Fusion'**

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PROTOCOL TITLE 'A Randomized Intra-Patient Controlled Trial of MagnetOsTM Granules vs. Autograft in Instrumented Posterolateral Spinal Fusion'

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
ASA	American Society of Anesthesiologists
BMP	Biphasic Calcium Phosphate
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CE	Conformité Européenne
CRF	Clinical Report Form
CRO	Clinical Research Organization
CT	Computer Tomography
CV	Curriculum Vitae
EU	European Union
EQ-5D	EuroQol Five Dimensions
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GEE	General Estimating Equations
HA	Hydroxy Apatite
IB	Investigator's Brochure
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsingcommissie (METC)
NFU	Dutch Federation of University Medical Centers; in Dutch: Nederlandse Federatie van Universitair Medische Centra
NRS	Numeric Rating Scale
NSAID	Non-Steroidal Anti-Inflammatory Drugs
ODI	Oswestry Disability Index
OLVG	Onze Lieve Vrouwe Gasthuis
PLF	Posterolateral Lumbar Fusion
PLIF	Posterior Lumbar Interbody Fusion
SAE	Serious Adverse Event
SOP	Standard Operating Procedure

Sponsor The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidizing party.

SPSS Statistical Package for the Social Sciences

Sv Sievert

TCP Tricalcium Phosphate

TLIF Transforaminal Lumbar Interbody Fusion

TM Trademark

UMC University Medical Center

VAS Visual Analogue Scale

Wbp Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)

WMO Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

SUMMARY

Rationale: Posterolateral spinal fusion is currently performed by using large amounts of autologous bone graft. Drawbacks of bone grafting include the need for an additional surgical procedure, limited supply, sub-optimal bone quality in osteoporotic patients and harvesting morbidity, which led to the development of numerous bone graft substitutes. Recently, we completed enrollment and one-year follow-up for a clinical trial to evaluate such a bone graft substitute (AxA study, METC number 13-192). The product, AttraX® Putty, is a bioresorbable tricalcium phosphate (TCP), mixed with a fast resorbing polymer carrier to improve surgical handling. The preliminary results are promising in terms of no adverse events related to the product and a first impression of similar fusion rates. However, especially in more challenging conditions, both the autograft and the material may resorb too fast and bone formation by induction was limited. Recently an improved version of the TCP granules has been developed, named MagnetOs™ Granules, which has shown favorable results especially of these resorption and induction characteristics in pre-clinical studies. MagnetOs™ Granules are CE-marked (2115660CE01) and received 510(k) clearance from the US Food and Drug Administration (K161859).

Objective: The primary objective is to demonstrate non-inferiority of MagnetOs™ Granules compared to autograft in instrumented posterolateral spinal fusion, in terms of efficacy and safety by means of an intra-patient model.

Study design: This study is designed as a multicenter, observer blinded, randomized, controlled non-inferiority trial with intra-patient comparisons.

Study population: 100 adult patients qualified for posterolateral spinal fusion in the thoracolumbar and lumbosacral region (T10-S2) will be recruited and enrolled in this study.

Intervention: According to a randomization scheme, one side of the spine will be grafted with the MagnetOs™ Granules and the other side with bone harvested from the iliac crest and local bone. The rest of the surgical procedure will be according to standard care.

Main study parameters/endpoints: The primary efficacy outcome is the rate of successful posterolateral spinal fusion after one year, assessed on CT-scans. Non-inferiority of the MagnetOs™ condition compared to the autograft condition will be assessed using a McNemar's test. The primary safety outcome is the number and nature of (serious) adverse events related to the surgical procedure compared to control populations from literature. Secondary outcomes are the comparison to its predicate (AttraX® Putty), relation between posterolateral fusion and interbody fusion after one-year, posterolateral spinal fusion rate after two years, relevance of iliac crest donor site pain and the incidence of long-term complication and relation with risk factors in the combined population of this study and the AxA study.

Nature and extent of the burden and risks associated with participation: Patient burden and risks are expected to be minimal. The first-year follow-up will be according to the

standard of care at the UMC Utrecht. Additional procedures for this study include the completion of short patient reported outcome measurements at five time points and, depending on the local follow-up protocol, a CT-scan at one-year follow-up and/or X-ray at two years follow-up. In case the one-year CT-scan shows a doubtful fusion or non-union in any of the relevant levels, an additional CT-scan will be made at two years follow-up. Based on pre-clinical investigations, and the results of the AxA study, the risk for inferior performance of MagnetOsTM Granules is expected to be minimal. Even if this appears to be the case, it will have minimal consequences for the patient as the other side of the spine will be fused with autologous bone graft and the spine is rigidly instrumented with screws and bars. All patients may benefit from the study in terms of reduced surgical time, since only half of the required bone graft will be harvested from the iliac crest.

1. INTRODUCTION AND RATIONALE

Spinal fusion is a surgical procedure frequently used for many spinal conditions requiring stabilization of the vertebral column, with or without correction of deformities. The primary surgical aim of this procedure is to establish bony fusion between one or more vertebral segments. Many fusion techniques are available ranging from anterior interbody fusion to posterior or posterolateral procedures and combinations of these. Especially in conditions of mechanical instability, the bony fusion is facilitated by rigid instrumentation of the vertebrae. Bony fusion will develop in time between the immobilized vertebrae when the bone surfaces are denuded and graft material is added. This process of fusion is a relatively slow process that takes months to years before complete fusion. Therefore, higher fusion rates are observed after one year as compared to six months. Autologous bone grafting (or autografting) with bone harvested from the iliac crest is the gold standard to achieve bony fusion. This is a reliable and well known technique for many indications.¹ Whether the bone formation after autografting is the result of direct bone formation from the graft, induction by the graft, or conduction of host bone remains a matter of debate.² It is a fact however, that alternatives like allograft and ceramics generally perform less good than autologous bone from the iliac crest. Bone morphogenic proteins (BMP-2, OP-1) were a popular alternative, however, these growth factors have been related to serious complications and may not perform as good as autograft.^{3,4} Especially when more extensive and challenging fusions are performed, large amounts of graft are required. This can be problematic since the amount of available bone graft from the iliac crest is limited and large quantities may lead to more morbidity.³ If extensive decompression is not required, fusion procedures on a single spinal level are nowadays often performed with less invasive techniques, combining interbody cages with relatively low amounts of bone graft and/or percutaneous pedicle screw instrumentation. The real value of an autograft bone substitute is therefore mainly for the more extensive surgical procedures where large amounts of autograft are required. Such cases include the treatment of multilevel spinal stenosis, primary or secondary scoliosis and other deformities. A graft substitute will reduce donor site morbidity and surgery time in those cases. At the same time, these conditions are less forgiving and require a graft substitute that has at least the same bone forming potential as the autograft. Consequently, the graft substitute has to perform like autograft in terms of both generating bone volume and bony fusion.

For decades, a suitable alternative to the autologous bone graft has been the subject of extensive research. Initially, allogeneic and xenogeneic bone grafts from a human donor and animal donor respectively were investigated. Although still very often applied, allogeneic grafts do not perform as good as autografts in many situations and they carry a theoretical

risk of disease transmission.⁵⁻⁷ Since the 1970s synthetic ceramics have been investigated as a more safe and predictable graft substitute. As most ceramics represent the anorganic mineral-phase of bone tissue, this strategy seems logical and attractive. Major concerns with ceramics are the brittleness and resistance to resorption and remodeling. An alternative is to use the organic-phase of donor bone, the so-called demineralized bone matrix (DBM). However, also this material has disadvantages of which unpredictable effectiveness is one of the major concerns.^{8,9} Since the 1990s, bone morphogenetic proteins (BMP's) have been introduced and applied at a large scale, especially in the United States. Most well-known are BMP-2 and BMP-7, which have shown effectiveness in several preclinical and clinical studies. However, these effects could only be accomplished when extremely high dosages were applied. These extreme doses may be the cause of the recently reported serious side effects.⁴

Since the late 1990s, a specific group of ceramics has been identified and developed that possesses osteoinductive activity in some animal models without the addition of any substance.^{10,11} The exact mechanism behind this characteristic has not yet been elucidated, but several important parameters like microporosity and surface characteristics have been identified.^{11,12} Furthermore, it has been shown that osteoinductive characteristics are associated with enhanced bone formation in several pre-clinical animal models.¹³ Such a ceramic has been commercialized under the name AttraX® Putty (NuVasive, San Diego, CA) and is investigated by us in a similar multicenter, randomized, controlled trial (AxA study, METC number 13-192). This study started in October 2013. The last patient was operated in July 2016 and one-year follow-up was completed in July 2017. A total of 108 patients were included and 100 patients were operated according to the study protocol. The current study will be according the same intra-patient design which has many advantages as explained below. The preliminary results of the AxA study indicate favorable results in terms of a perceived similarity between autograft and AttraX® Putty and the absence of product related (serious) adverse events. During the AxA study we did observe that, especially in challenging conditions with long fusion trajectories, both the autograft and AttraX® Putty placed around the instrumentation and in the posterolateral gutters resorbed too fast and intertransverse fusion did not occur, or only between the facet joints. That observation was confirmed by the steep reduction in graft density between 3 and 6 months observed with DEXA-scanning.

Recently the composition and manufacturing of AttraX (which was originally developed by Progentix Orthobiology, the Netherlands) has been changed by the same research group (Prof. de Bruijn and Dr. Yuan, Kuros Biosciences BV, The Netherlands). These changes involve a higher hydroxyapatite (HA) content which causes a lower resorption rate, and a

surface modification which has shown more favorable osteoinductive activity in several studies, as well as less rapid resorption compared to autologous bone.¹⁴⁻¹⁸ In a preclinical posterolateral spinal fusion study in sheep, the fusion rate was comparable to autograft and not influenced by adding a binder.¹⁶ This new version has received a CE-mark (number 2115660CE01) followed by 510(k) clearance from the US Food and Drug Administration (FDA) under the name MagnetOsTM Granules and is produced by Kuros Biosciences BV.

The aim of the current study is to clinically investigate MagnetOsTM Granules as an alternative to autologous bone graft in adult patients qualified for instrumented posterolateral fusion of the thoracolumbar or lumbosacral spine by means of an intra-patient model. This patient population will benefit most from such a graft substitute. We expect that MagnetOsTM Granules will perform as good as autograft, while eliminating the morbidity and increased surgical time associated with harvesting of autologous bone from the iliac crest.

2. OBJECTIVES

2.1 Primary objectives

The primary objective of this study is to demonstrate the non-inferiority of MagnetOsTM Granules as compared to autologous bone graft in instrumented posterolateral fusion of the thoracolumbar and lumbosacral spine, in terms of efficacy and safety.

1. Efficacy will be based on the posterolateral spinal fusion rate assessed at one-year CT-scans;
2. Safety will be evaluated by documenting the number and nature of all (serious) adverse events that may in any way be related to the surgical procedure. The complication rate will be compared to the rate in control populations from literature.

2.2 Secondary objectives

1. To compare the quantitative primary and secondary results of MagnetOsTM Granules to the results of Attrax® Putty from the AxA study (unpaired);
2. To investigate the relation between successful posterolateral fusion and interbody fusion, assessed at the one-year CT-scans used for the primary objective;
3. To compare the posterolateral spinal fusion rate of both MagnetOsTM Granules and autograft at one year with the fusion rates after two years, by using CT-scans;
4. To investigate the effect of blinding on perceived donor site pain, based on outcome measures reported by patients unblinded to the iliac crest donor site in comparison to these outcomes of the blinded patients from the AxA study;
5. To combine the study populations of the current study and the AxA study to investigate a) adjacent segment disease, in relation to length of construct and sagittal balance and b) risk factors for failures.

2.3 Rationale for secondary objectives

Besides the primary research question on efficacy and safety, this study gives the opportunity to investigate very relevant scientific questions that can only be answered in human patients. Furthermore, combining the study data with the data obtained from the AxA study increases the power to study phenomena that occur less frequently.

1. Comparison of the quantitative results of MagnetOsTM Granules to Attrax® Putty

Since both the study designs are the same and have the same in- and exclusion criteria, it will be possible to compare the groups in a standard unpaired fashion. We did not power this comparison since it is a secondary aim, but the findings will be indicative with a considerable

sample size of 200 individuals (each with autograft at one side of their spine and MagnetOs™ Granules (n=100) or AttraX® Putty (n=100) at the other side).

2. Relation between posterolateral fusion and interbody fusion

Since this study will not exclude interbody fusions, and the effect of such interbody fusions on the posterolateral fusion masses is unknown, it is desired to determine the statistical relation between the posterolateral and interbody fusions.

3. Fusion rate after 2 years

There are indications that the percentage bony fusion continues to increase between 1 and 2 years.²⁶⁻²⁸ Whether this is graft related or a result of immobilization due to instrumentation is unclear. Nevertheless, it is important to determine the long-term fusion potential and behavior of MagnetOs™ Granules and autograft, and to compare these conditions. To limit the number of CT-scans at 2 years follow-up, only those patients that scored a doubtful fusion or non-union in any of the relevant levels at the one-year CT-scan will undergo an additional CT-scan.

4. Relevance of donor site pain

Although iliac crest donor site morbidity is often reported by patients, the relevance of donor site pain is disputable when the bone is harvested via the same incision as used for the spinal fusion.³ The preliminary results of the AxA study indeed indicate that there is no relation between the real donor site and the iliac crest that is indicated by blinded patients. To determine if the often reported high incidence of donor site pain can be explained by the knowledge of the patient where the bone graft was taken, we will not blind the patients for the donor site in this study. The outcomes measures reported by patients unblinded to the iliac crest donor site will be compared to the outcomes of the blinded patients from the AxA study.

5. Combine the study populations of the current study and the AxA study

To combine the study data of both the current study and the AxA study will allow investigations on more infrequent phenomena on a relatively large population. Such a phenomenon is for example adjacent segment disease. This is a longterm complication where the adjacent joints and intervertebral disc may wear faster and cause degenerative disease. The occurrence of adjacent segment disease may be related to length of the construct and restoration of the sagittal balance. The occurrence of typical complications like infection and pseudoarthrosis can be related to risk factors like comorbidities, smoking, length of instrumented segment and so on.

3. STUDY DESIGN

This study is a multicenter, observer blinded, randomized, intra-patient controlled, non-inferiority trial with intra-patient comparisons, which is initiated and will be coordinated by the department of Orthopaedics from the UMC Utrecht, the Netherlands. A multicenter design is necessary to include the required number of patients (n=100) in an acceptable and feasible timeframe. Due to the experience with the AxA trial we have an established cooperation with the other centers which will make implementation in all centers relatively easy.

Adult patients qualifying for instrumented posterolateral spinal fusion of one to six levels in the thoracolumbar and lumbosacral region (T10-S2) with the use of autograft will be recruited by their treating orthopaedic surgeon or his/her assistant. After obtaining informed consent, they will be operated locally according to normal planning and the surgical techniques suggested in Appendix B. Per-operatively, after instrumentation of the spine and preparation of the bilateral trajectory for grafting, the randomized allocation of the graft type (left or right) will be disclosed. So in each patient, each side of the fusion trajectory will be one of the two conditions: the **autograft condition**, a combination of local bone obtained from decompression and/or preparation for fusion (facetectomy and denudement), supplemented with at least 50% corticocancellous bone harvested from the iliac crest, or the **MagnetOs condition**. Allocation of the conditions will always be to only one side of the spine, also when multiple levels are incorporated. Corticocancellous bone will be taken from only one iliac wing at the side of the autograft condition, unless this side is inappropriate due to previous harvesting or anatomical reasons. If possible, harvesting is done via the same posterior incision for spinal fusion. In case of an additional interbody fusion procedure, the cage is preferably filled with local autograft bone and positioned before opening of the randomization envelope. If the surgeon decides to use iliac crest bone for this device, the cage can only be inserted after the randomization and related harvesting side has been disclosed. All patients will be evaluated radiologically and clinically at several time points pre- and postoperatively (see Figure 3.1), and all (serious) adverse events that in any way may be related to the procedure will be carefully documented and evaluated to meet the objectives described in Chapter 2.

The expected duration of the study is approximately 4 years from the commencement of patient enrollment; enrollment is expected to take two years, followed by two years follow-up. After completion of one-year follow-up, the analysis for the primary outcomes will be done by the investigators at the UMC Utrecht.

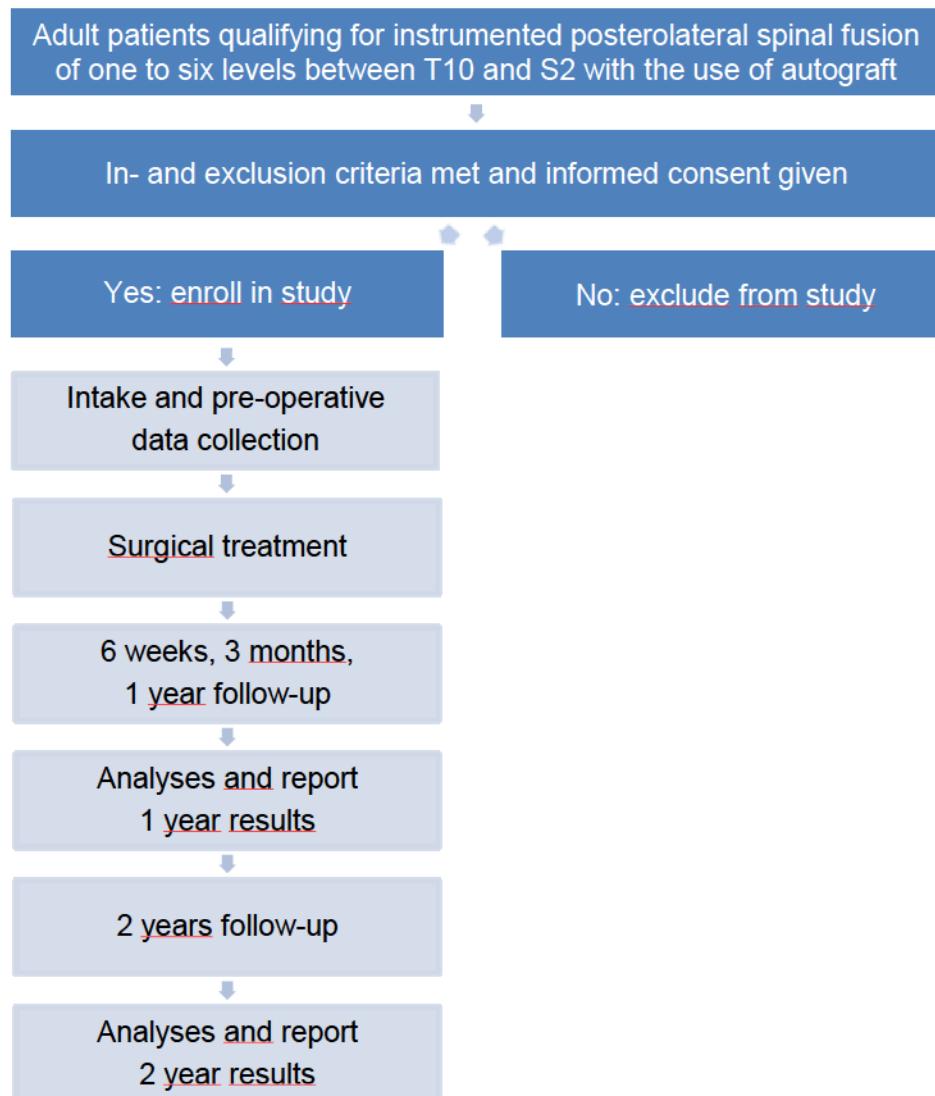


Figure 3.1: Schematic overview of study design.

Rationale for intra-patient design

To investigate the efficacy of a bone graft substitute like MagnetOs™ Granules in the patient group that will benefit most from it, is challenging as these patients and surgeries are quite unique. The types of fusion in terms of location and number are highly variable and difficult to compare. Also, several surgical techniques are applied in addition to the posterolateral fusion at various levels. For example, posterior lumbar interbody fusion (PLIF) or transforaminal lumbar interbody fusion (TLIF). The solution to these impediments is to use a model where the patient is its own control. This is a recognized option in posterolateral spinal fusion investigations, because the right and left side of the spine are subject to similar procedures but physically separated.^{29,30} Moreover, the assessment of spinal fusion in terms of bone continuity and bone volume of both sides can be done independently. By using an intra-patient comparison model, the number of patients required for sufficient study power will be even less than half the number needed for traditional inter-patient comparisons. This is

because inter-patient variability of confounders for bony fusion is irrelevant with this approach. Finally, an important ethical advantage of this model is that the patient always receives the gold standard treatment for posterolateral fusion at one side of the spine, and for interbody fusion as well when an additional PLIF or TLIF procedure is performed. When the bone graft substitute would unexpectedly fail to establish a bony fusion, the implications will be much less severe because the contralateral side will have the gold standard treatment and a unilateral fusion of the spine is sufficient.

A frequently mentioned disadvantage of an intra-patient comparison model can be a disturbing effect of one condition on the other.³¹ This is definitely a concern when the stabilizing effect of fusion on one side of the spine might affect the other side. However, when spinal instrumentation is used, motion is drastically limited and not likely to be a factor of importance. Another disadvantage of an intra-patient model is when bioactive factors like BMPs are used, as these may influence the contralateral site. However, this is not a concern in the case of the current ceramic material, as it does not contain any bioactive additives. The main concern with the intra-patient model is that it does not allow assessment of the clinical effect of the intervention. This is a very important limitation that should be recognized. However, it is not an issue with respect to our research questions. The clinical outcome of spinal fusion is a matter on itself, which actually does not really reflect the functional and radiological presence of fusion especially not in the first years.³² Therefore, this study aims to evaluate the efficacy of in terms of percentage bony fusion, assessed at one year CT-scans.

4. STUDY POPULATION

4.1 Population (base)

Adult patients, both males and females, qualifying for instrumented posterolateral spinal fusion of one to six levels between T10 and S2 with the use of autograft will be recruited by their treating orthopaedic surgeon or his/her assistant at the UMC Utrecht, OLVG Amsterdam, St. Antonius Hospital Utrecht, Rijnstate Arnhem or Amphia Hospital Breda. All patients will have had at least one conservative treatment attempt prior to enrollment. Based on the sample size calculation described in Paragraph 4.4 and Appendix A, the total number of patients to be enrolled is 100. Based on our experience with the AxA study, patient enrollment is expected to take two years.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a patient must meet all of the following criteria:

- To be treated with instrumented posterolateral thoracolumbar spinal fusion with the use of iliac crest bone, with or without additional posteriorly inserted interbody devices (PLIF, TLIF), because of (1) deformity, (2) structural instability and/or (3) expected instability as a result of decompression for spinal stenosis;
 1. Deformity is defined as a scoliosis in the coronal plane of $>20^\circ$ and/or a sagittal balance disturbance according the SRS/Schwab classification³³ on standardized standing full spine radiographs;
 2. Preoperative instability is defined as a progressive angular deformity or spondylolisthesis in standing radiographs;
 3. Decompression for spinal stenosis is done in the occurrence of radiological evidence of stenosis on MRI and clinical leg and/or back pain with one or more of the following phenomena: radiculopathy, sensory deficit, motor weakness, reflex pathology or neurogenic claudication.
- Non-responsive to at least 6 months of non-operative treatment prior to study enrollment;
- Fusion indicated for one to six levels in the T10 to S2 region. In case of vertebral osteotomies (PSO or VCR) the osteotomized segment will not be included in the assessment of the fusion rate;
- Willing and able to understand and sign the study specific Patient Informed Consent;
- Skeletally mature between 18 and 80 years of age.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Any previous surgical attempt(s) for spinal fusion (revision surgery) of the intended segment(s);
- Previous treatments that compromise fusion surgery like irradiation;
- Previous autologous bone grafting procedures that compromise the quality and amount of iliac crest bone grafting;
- Indication for spinal fusion because of an acute traumatic reason, like a spinal fracture;
- Active spinal and/or systemic infection;
- Spinal metastasis in the area intended for fusion;
- Systemic disease or condition, which would affect the subjects ability to participate in the study requirements or the ability to evaluate the efficacy of the graft (e.g. active malignancy, neuropathy, pregnancy);
- At risk to be non-compliant e.g.: (recently treated for) substance abuse, detainee, likely to immigrate
- Participation in clinical trials evaluating investigational devices, pharmaceuticals or biologics within 3 months of enrollment in this study;
- Female patients who intend to be pregnant within 1.5 year of enrollment in the study;
- Body mass index (BMI) larger than 36 (morbidly obese);
- Being expected to require additional surgery to the same spinal region within the next 6 months;
- Current or recent (<1yr) corticosteroid use equivalent to prednisone ≥ 5 mg/day, prescribed for more than 6 weeks.

4.4 Sample size calculation

The primary objective of this study is to demonstrate that treatment with MagnetOsTM Granules is non-inferior to autograft to obtain bony fusion in the spine. The primary outcome is the rate of successful posterolateral bony fusion after one year, assessed at CT-scans. Fusion will be scored binomially and non-inferiority of fusion in the MagnetOs condition will be assessed using a McNemar's test.

Based on literature^{6,28-30,34} and the preliminary results of the AxA study, we estimate a fusion percentage of 50% for unilateral grafting after one year, and a 70% concordance between the left and right side of the fusion trajectory (i.e. both sides fused or non-fused). The non-

inferiority margin is set at an absolute 15% less effectiveness in the MagnetOs condition after one year, which corresponds with 15% less posterolateral sides that are judged as fused. In other words, the rate of fused posterolateral sides in the MagnetOs condition should be more than 35% if the autograft condition shows 50% fusion. We argued that the percentage of the iliac crest graft related complications, ranging from 5% major complications to 40% minor complications, may justify 15% less fusion in a condition that eliminates these complications.

Based on a 1:1 paired assignment of MagnetOsTM Granules to the control condition with autograft, a power of 80%, a one-sided alpha of 0.05 and the non-inferiority margin of 15%, the required sample size for the primary outcome is 84 patients (see Appendix A). We assume that approximately 15% of the included patients will not be evaluable after one year. The intra-patient analyses are not possible when the fusion area has to be revised or when a deep wound infection has occurred and considerable amounts of graft material have to be removed (expected percentage 2-5%). Other failures are lost to follow-up or bad scan quality. Therefore, the total number of patients to be enrolled will be 100.

5. TREATMENT OF SUBJECTS

As explained in the introduction, autologous bone grafting is the gold standard to achieve bony fusion in (instrumented) spinal fusion procedures. In this study, according to a randomization scheme, one side of the spine will be grafted with the synthetic ceramic material MagnetOsTM Granules instead of local bone mixed with bone harvested from the iliac crest. The rest of the surgical procedure will be according to standard care. The investigational product is described more detailed in the next Chapter; the treatment procedure and follow-up are described in Chapter 7.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product

MagnetOsTM Granules is a synthetic ceramic that has a CE-mark (2115660CE01) as well as FDA 510(k) clearance (K161859). It is comprised of calcium phosphate granules with a tricalcium phosphate (β -TCP) and hydroxyapatite (HA) ratio of 70/30. The calcium phosphate granules have shown to be bioactive, osteoconductive and osteoinductive.¹¹

MagnetOsTM Granules is intended for use as a bone void filler for voids and gaps of the skeletal system that are not intrinsic to the stability of the bony structure, and not too large that they would fail to heal spontaneously in the surgeon's opinion. The device may be combined with autogenous bone, blood, platelet-rich plasma, and/or bone marrow. In load-bearing situations, MagnetOsTM Granules is to be used in conjunction with internal or external fixation devices.

6.2 Summary of findings from non-clinical studies

Biocompatibility of MagnetOsTM Granules has been established through testing and evaluation per ISO 10993 standards for permanent implant contacting bone/tissue. The material was found to be non-cytotoxic, non-sensitizing, non-irritating, non-genotoxic, and non-toxic.

To test device performance, MagnetOsTM Granules has been compared to predicates including AttraX Granules (a.k.a. CuriOsTM), VitossTM BA (Orthovita) and ActifuseTM ABX (Apatech) in a series of benchtop and animal studies. Benchtop degradation tests show that MagnetOsTM Granules are resorbable. The resorption rate of MagnetOs is situated within the range of other commercially available calcium phosphate bone void fillers. From the quickest to slowest resorbable material: Vitoss>AttraX>MagnetOs>Actifuse ~ Pro-Osteon ~ OsSatura. Testing of the structure of the calcium phosphate components using mercury intrusion porosimetry showed that MagnetOsTM Granules have a total porosity of 70±15%. The total surface area of MagnetOsTM Granules is larger than that of CuriOsTM, VitossTM BA and ActifuseTM ABX. These surface features have shown to be important in promoting bone formation.

Ectopic implantation in a dog intramuscular model showed the osteoinductive potential and biocompatibility of MagnetOsTM compared to OsOpia (REGEDENT AG) and CuriOsTM. After 12 weeks implantation, no necrosis and inflammation were seen in any of the explants. MagnetOsTM gave rise to earlier bone formation than CuriOsTM and OsOpia^{14,15} while bone%

and cellular activity on the different microstructures were equivalent for all groups at 12 weeks.

Two preclinical studies in spine posterolateral models showed efficacy of MagnetOs™ Granules. In a sheep model of instrumented posterolateral spinal fusion, of MagnetOs™ Granules with or without a binder showed to have similar fusion rates at different time points compared to autograft. At 12 weeks, the fusion rate assessed by manual palpation and histology showed a high fusion performance (see table 1). The tissue responses were similar for all implanted bone grafting materials. Material resorption and bone formation were quantified by histomorphometry. An equivalent amount of bone was formed and a slower resorption of MagnetOs™ Granules was observed, as compared to CuriOs™ granules. Distant organs and blood analyses confirmed the lack of systemic toxicity.¹⁶

Table 1. Fusion by histology (instrumented Sheep spine posterolateral fusion)

U=unilateral fusion; B=bilateral fusion; FR=Fusion rate. BCP= MagnetOs

	6 wks			12 wks			26 wks		
	U	B	FR	U	B	FR	U	B	FR
Autograft	1/5	0/5	10%	1/6	4/6	75%	1/5	4/5	90%
BCP	0/5	0/5	0%	1/6	5/6	92%	0/5	5/5	100%
BCP + Binder	0/5	0/5	0%	2/6	4/6	83%	1/5	4/5	90%

As an autograft extender, MagnetOs™ Granules achieved similar fusion rates compared to autograft alone in a rabbit bilateral un-instrumented posterolateral spinal fusion. At 6, 9 and 12 weeks, the animals were essentially equivalent based on all end point evaluations (manual palpation, radiography, micro-CT, histology). Progression of fusion was also seen for all end points at 6, 9 and 12 weeks. Mechanical testing (flexion-extension and lateral bending) at 12 weeks demonstrated fusion of the treated level, which was comparable to the autograft group and in line with the manual palpation results. Histology and histomorphometry versus time demonstrated the normal features of bone healing and progression to spinal fusion. An osteoconductive response was noted at 6 weeks with newly formed bone formation both on and between the resorbing calcium phosphate mineral as well as with the residual autograft particulate. The normal concert of new bone formation, implant resorption and remodeling occurred with time as the newly formed bone remodeled together the material resorption and normal marrow spaces developing to mature fusion space at 12 weeks. No adverse reactions were observed.

Table 2. Fusion by histology (Rabbit spine posterolateral fusion)

	Histological fusion		
	6 weeks	9 weeks	12 weeks
Autograft(AG)	0% fus	20% fus	60% fus
MagnetOs TM Granules/AG	10% fus	20% fus	70% fus
MagnetOs TM Putty/AG	10% fus	20% fus	60% fus

6.3 Summary of findings from clinical studies

No clinical investigations of MagnetOsTM Granules have been performed to-date.

6.4 Summary of known and potential risks and benefits

Spinal fusion with a synthetic bone graft substitute is a widely available surgical treatment option for patients with a spinal deformity. However, there are some short and long-term risks that one has to be aware of.

Short term risks of a bone graft substitute include, but are not limited to:

- Migration of the device from the site of implantation
- An allergic reaction to the implanted material

Long term risks of a bone graft substitute include, but are not limited to:

- Failure to promote bone fusion
- Excessive bone growth, or bone growth in unwanted locations

As mentioned above, MagnetOsTM Granules has shown in preclinical animal models to result in increased fusion potential and quicker fusion than similar commercially available materials. Potential benefits in the clinical setting might include higher fusion rates, and faster fusion times than other synthetics, as well as lower complications than autograft, allograft, or protein-based treatment options. This study is designed to show non-inferiority of MagnetOsTM Granules as compared to autograft for posterolateral spinal fusion as well as to determine potential risks in the clinical setting.

6.5 Description and justification of route of administration and dosage

In this study, MagnetOsTM Granules will be used as an alternative to autograft for instrumented posterolateral fusion of the thoracolumbar or lumbosacral spine, to evaluate its efficacy and safety. After instrumentation and preparation of the bilateral trajectory for grafting, the recommended volume of 8-10cc of per spinal level will be placed at the randomized allocation side, without the addition of autogenous bone, blood, plasma or bone

marrow. Close contact with vital bone is important for its function as a bone regeneration material. The surgeon will place the granules around the instrumentation and in the decorticated lateral gutters, bridging the dorsal surfaces of the transverse processes, facets and laminae, without destruction of the granules structure.

6.6 Product storage and accountability

MagnetOs™ Granules will be provided by the subsidizing party in commercially available packages of 5cc (vials in a pouch). The product will be stored at each participating site according to the recommended storage conditions. Shipment and distribution records as well as product accountability logs will be retained. At the end of the study, unused products will be returned to the subsidizing party.

7. METHODS

7.1 Study outcomes

7.1.1 Primary study outcomes

1. Posterolateral spinal fusion rate at one-year follow-up (assessed on CT-scans);
2. Rate of (serious) adverse events that are related to the procedure in any way, and their potential relation with MagnetOsTM Granules.

7.1.2 Secondary study outcomes

1. Comparison of the quantitative primary and secondary results of MagnetOsTM Granules to the results of AttraX® Putty from the AxA study (unpaired);
2. Relation between successful posterolateral fusion and interbody fusion at one-year follow-up (assessed on CT-scans);
3. Posterolateral spinal fusion rate at two years follow-up compared to the fusion rate at one-year follow-up (assessed on CT-scans);
4. Effect of blinding on perceived donor site pain;
5. Incidence of long-term complications and relation with risk factors in the combined population of this study and the AxA study.

7.1.3 Other study outcomes

This study provides the opportunity to further investigate several outcomes in a cohort of 100 spondylodesis patients, not related to the comparison of MagnetOsTM Granules and autograft.

1. Changes in EQ-5D-5L, ODI and VAS pain scores over time;
2. Relation between clinical outcomes and fusion status at one-year follow-up;
3. Satisfaction with treatment;
4. Effect of nicotine use on fusion;
5. Effect of NSAID use on fusion.

7.2 Randomization, blinding and treatment allocation

Randomization for the allocation of the graft type will be performed with use of a computerized random-number algorithm. The randomization scheme will be maintained at the UMC Utrecht by the central principal investigator and study coordinator. The orthopaedic surgeon will be blinded to the allocation side of MagnetOsTM Granules, and thereby the contralateral allocation of autograft, as long as possible. He/she will receive an envelope containing the randomization of the patient which should be opened during surgery just

before the graft is needed (see suggested surgical technique, Appendix B). The iliac bone graft has to be taken from the same side where the autograft will be applied for posterolateral fusion according to the randomization. This will not be communicated to the patient. Whenever possible, bone harvesting will be done without making an additional skin incision.

In case an intra-operative decision is made to perform something different than what was intended for study enrollment, like for example to use local bone graft only or an uninstrumented spinal fusion, the patient will be considered a per-operative withdrawal and will not be treated with MagnetOs™ Granules. These patients will be replaced as described in paragraph 7.5.

7.3 Study procedures

Figure 7.1 illustrates the timing of the different study procedures that are described below. Procedures that are not part of standard care in one or more participating centers are marked bold.

All study data will be entered in electronic Case Report Forms (eCRFs) using Castor EDC. To limit missing data and ensure timely completion, the patient questionnaires are preferably filled out digitally by using the email function in Castor EDC. If patients prefer paper questionnaires, they should be completed at the outpatient clinic.

De-identified copy of all corresponding images will be send to the principal investigator at the UMC Utrecht.

Table 7.1: Timing of study procedures and data collection. Procedures that are not part of standard care in one or more participating centers are marked bold. Abbreviations: d = days, wks = weeks, mos = months, yr(s) = year(s).

CRF form	Preop	Perop	1-5 d	6 wks (±14d)	3 mos (±21d)	1 yr (+3mos)	2 yrs (±2mos)
	A	B	C	D	E	G	H
Demographics	x						
Baseline data							
Treatment details		x					
Clinical evaluation	x		x	x	x	x	x
X-rays	x		x	x	x	x	x
CT-scan						x	x ¹
Patient questionnaires	x			x	x	x	x

¹ Only applies to patients that scored a doubtful fusion or non-union in any of the relevant levels at the one-year CT scan (see Appendix C)

7.3.1 Baseline data

After enrollment (see Chapter 10 for the recruitment and consent procedure), a preoperative baseline evaluation will be performed and the following data will be collected (CRF A):

- Month and year of birth
- Gender
- Smoking
- Pre-operative diagnosis
- Prior treatment to affected spinal levels
- Concurrent medical conditions
- ASA classification
- Disease related medication use (including NSAID use)
- Questionnaires
 - EQ-5D-5L
 - ODI
- VAS pain score
 - Back pain related to diagnosis
 - Leg pain related to diagnosis
 - Pain in left iliac crest
 - Pain in right iliac crest
 - Pain drawing

The EQ-5D-5L is a standardized instrument for use as a measure of health outcome. Applicable to a wide range of health conditions and treatments, it provides a simple descriptive profile and a single index value for health status that can be used in the clinical and economic evaluation of health care as well as population health surveys. The Oswestry Low Back Pain Disability Index (ODI) is an evaluation questionnaire related to patient perception of the effect of his/her current low back pain on activities of daily living. A Visual Analogue Scale (VAS) will be used to record back pain, leg pain and iliac crest pain at the left and right side at all evaluation points. As a supplement, patients will be asked to make a pain drawing to get a graphic representation of the pain and its physical distribution.

7.3.2 Surgical and postoperative procedures

Patients will be operated according to standard surgical procedures for instrumented posterolateral fusions between T10 and S2, with the only exception that one side of the spine will be grafted with the synthetic ceramic material MagnetOs™ Granules instead of bone harvested from the iliac crest. A recommended surgical technique is provided in Appendix B. In advance, the participating orthopaedic surgeons of each center will receive instructions on appropriate use of MagnetOs™ Granules from the principal investigator. Any questions that may arise concerning the surgical aspects of using MagnetOs™ Granules or autograft will be answered by the principal investigator.

Standard prophylactic antibiotics will be given for at least 24 hours, according the local surgical guidelines of each center. A thrombosis prophylaxis will be used according to the surgeons' preference. If preferred by the surgeon, patients will receive a restraining brace or orthosis postoperatively in order to protect the spine from (excessive) movements. Mobilization will be encouraged on the first postoperative day; physiotherapy will be offered during hospitalization and ambulatory if indicated.

According to standard care, standing anteroposterior and lateral radiographs will be taken postoperatively during hospitalization.

During surgery and hospital admission, the following information will be collected (CRF B):

- Date of surgery
- Surgeon's name
- Surgical technique
 - Instrumented spinal levels
 - Levels of decompression
 - Performance of vertebral osteotomy
 - Use of interbody device
 - Insertion side of TLIF
 - Additional surgical procedures
- With the use of a 20ml syringe an estimation of the volumes (in mL) of:
 - 1) obtained local bone and 2) obtained iliac crest bone and 3) the ratio of local bone / iliac crest bone used for posterolateral fusion
 - Local bone (or mixed autograft) applied per level for interbody fusion
 - (Mixed) autograft used per posterolateral fusion level

- Estimation of volume of MagnetOsTM Granules per posterolateral fusion level (in cc)
- Iliac crest side used for bone harvesting (right/left)
- Iliac bone harvested via same incision (yes/no)
- Additional time required for bone graft harvesting (minutes)
- Allocation of MagnetOsTM Granules (left/right)
- Surgical time (skin to skin and total anaesthesia time (begin and end of sedation)
- Estimated blood loss (cc)
- Use of vancomycine powder
- Intra-operative complications
- Postoperative X-rays
- Date of discharge
- Disease related medication use (including NSAID use)

7.3.3 Planned follow-up moments

The subsequent follow-up moments will take place at the outpatient clinic and are based on the standard care at the UMC Utrecht. The data described below will be collected at 6 weeks, 3 months, 1 year and 2 years postoperatively. The questionnaires, CT-scan at 2 years follow-up in case of no or doubtful fusion at 1 year follow-up, and depending on the local follow-up protocol the CT-scan at one year follow-up and/or X-ray at two years follow-up, are extra for this study. In addition to the baseline questionnaires, treatment satisfaction will be assessed with a Numeric Rating Scale (NRS).

6 weeks (±14 days; CRF C)

- Complications / (serious) adverse events
- Disease related medication use (including NSAID use)
- Questionnaires
 - EQ-5D-5L
 - ODI
 - NRS satisfaction
- VAS pain score
 - Back pain related to diagnosis
 - Leg pain related to diagnosis
 - Pain in left iliac crest

- Pain in right iliac crest
- Pain drawing
- Imaging
 - Standing anteroposterior and lateral radiographs
- Radiologic evaluation by treating surgeon (indications for failure or deformation / instability)

3 months (\pm 21 days; CRF D)

- Complications / (serious) adverse events
- Disease related medication use (including NSAID use)
- Questionnaires
 - EQ-5D-5L
 - ODI
 - NRS satisfaction
- VAS pain score
 - Back pain related to diagnosis
 - Leg pain related to diagnosis
 - Pain in left iliac crest
 - Pain in right iliac crest
 - Pain drawing
- Imaging
 - Standing anteroposterior and lateral radiographs
- Radiologic evaluation by treating surgeon (indications for failure or deformation / instability)

1 year (12-15 months; CRF F)

- Complications / (serious) adverse events
- Disease related medication use (including NSAID use)
- Questionnaires
 - EQ-5D-5L
 - ODI
 - NRS satisfaction
- VAS pain score
 - Back pain related to diagnosis
 - Leg pain related to diagnosis
 - Pain in left iliac crest

- Pain in right iliac crest
- Pain drawing
- Imaging
 - Standing anteroposterior and lateral radiographs
 - CT-scan
- Radiologic evaluation by treating surgeon (indications for failure or deformation / instability)
- Fusion assessment based on CT-scan by treating surgeon per level and per side in three planes, without the distinction between graft-related or non-graft-related fusion as described in Appendix C.

2 years (22-26 months; CRF G)

- Complications / (serious) adverse events
- Disease related medication use (including NSAID use)
- Questionnaires
 - EQ-5D-5L
 - ODI
 - NRS satisfaction
- VAS pain score
 - Back pain related to diagnosis
 - Leg pain related to diagnosis
 - Pain in left iliac crest
 - Pain in right iliac crest
 - Pain drawing
- Imaging
 - Standing anteroposterior and lateral radiographs
 - CT-scan of all patients with a doubtful fusion or non-union after 1 year, as assessed by the blinded observer
- Radiologic evaluation by treating surgeon (indications for failure or deformation / instability)
- Fusion assessment based on CT-scan by treating surgeon per level and per side in three planes, without the distinction between graft-related or non-graft-related fusion as described in Appendix C.

7.4 Radiologic evaluation

7.4.1 X-rays

The regular standing anteroposterior and lateral radiographs will be evaluated locally by the treating surgeon. Indications for failure like material breakage or loosening and deformation or instability like osteolysis will be recorded, as well as indications for bone bridging.

7.4.2 CT-scans

The CT-scans will be reviewed both locally by the treating surgeon and centrally by an assessment panel consisting of at least one spine surgeon and an uninvolved specialist. This panel will be blinded to the treatment allocation and perform the assessments independently on de-identified CT-scans (see Appendix C).

Each instrumented spinal level, that is not part of an osteotomized segment, will be unilaterally assessed for posterolateral fusion according the protocol, based on literature³⁵⁻³⁷ and experience from the AxA study. This grading system consists of three categories:

1. “Fusion” is defined as a continuous bony bridge from one vertebra to the other, in absence of any secondary signs of nonunion, such as fracture or loosening of the screws or rods (= 2 points);
2. “Doubtful fusion” indicates doubts about continuity or quality of the bony bridge (= 1 point);
3. “No fusion” indicates definite discontinuity or lack of the fusion mass, as well as obvious indications of mobility like material failure or apparent pseuocoarthrosis (= 0 points).

Assessemnt of posterolateral fusion is scored in the coronal, sagittal and axial plane. A segment will be regarded fused if at least one plane shows definite fusion (2 points) and at least one other plane a doubtful fusion (at least 1 point). Interbody fusion will be assessed in two planes and scored similarly.

The final assessment panel will score two locations of posterolateral fusion separately: between the transverse process and >1mm lateral or above the rod (graft related) and directly around the rod or fusion of the facet joint (probably not graft related). See Appendix C for a more detailed description of the CT-assessment for the primary and secondary outcomes.

The CT-scans will also be evaluated for the volume of fusion bone, signs of heterotopic ossification (i.e. extension of fusion mass beyond the intended fusion level) and if possible, the presence of graft remnants will be recorded.

7.5 Withdrawal of individual patients

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The investigators can decide to withdraw a patient from the study for urgent medical reasons. In case an intra-operative decision is made to perform something different than what was intended for study enrollment like for example to use local bone graft only or an uninstrumented spinal fusion, the patient will be considered a per-operative withdrawal and will not be treated with MagnetOs™ Granules. Protocol violations where the (surgical) procedure or follow-up (e.g. timing of one-year CT-scan) will influence the primary outcome will be regarded individually and may lead to withdrawal.

7.6 Replacement of individual subjects after withdrawal

Per-operative withdrawals will be replaced. Patients who are withdrawn or lost to follow-up post-operatively, i.e. after the treatment with MagnetOs™ Granules, will not be replaced in this study, unless the anticipated drop-out rate of 15% is exceeded.

7.7 Follow-up of subjects after withdrawal

All data collected until withdrawal will be used for the analyses as described in the protocol, unless the patient made an explicit objection. All withdrawn patients will be followed according to standard care.

7.8 Failures

Patients that need revision surgery that influences the fusion, will be considered failures and only excluded from the primary analysis and secondary outcomes related to the performance of MagnetOs™ Granules. This because the treatment effect on one side of the spine cannot be distinguished from the effect on the other side, so intention to treat analyses are not possible. Patients registered as failure will not be replaced, as the total number of patients takes this into account. Follow-up will continue according to the study protocol.

7.9 Premature termination of the study

All (serious) adverse advents that can be related to the surgical procedure in any way will be reported to the principal investigator as described in Chapter 8 and evaluated. If a relation to the performance of MagnetOsTM Granules is suspected, the allocation side can be disclosed. These findings will be discussed with the METC.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 AEs and SAEs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable medical experience occurring to a subject during the study, whether or not considered related to MagnetOsTM Granules. All adverse events that can be related to the surgical procedure that are observed by the participating investigators or their staff, or reported by the subject between or at the follow up visits, will be recorded in the Case Report Form. An overview and analysis of all these adverse events will be included in the final study report. AEs that are related to previous known or unknown diseases, or conditions that cannot be related to the procedure (like accidents or other interventions), will not be recorded.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that:

- Results in death;
- Is life threatening (at the time of the event);
- Requires hospitalization or prolongation of existing inpatients' hospitalization;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect; or
- Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission for other reasons will not be considered as a serious adverse event.

All SAEs that can be related to the surgical procedure that are observed by the local principal investigators or their staff, or reported by the subject between or at the follow up visits, will be recorded in the Case Report Form. The investigator will report all these SAEs to the sponsor within 24 hours after obtaining knowledge of the events by sending the SAE form. SAEs that are related to a previous known or unknown disease, or conditions that cannot be related to the procedure (like accidents or related to other interventions) will not be recorded.

The sponsor will report all reported serious adverse events through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.3 Follow-up of adverse events

All reported AEs will be followed until they have abated, or until a stable situation has been reached or until the last study visit. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

9. STATISTICAL ANALYSIS

The detailed statistical methods and justification are described in Appendix A.

9.1 Primary efficacy analysis

For the primary efficacy analysis, a comparison will be made between the fusion performance of the MagnetOsTM condition and the autograft condition, scored from the one-year (12-15 months) CT-scans according to the method described in Appendix C. Patients that required revision surgery influencing the fusion will be excluded from this analysis. In addition, if one side of a fusion-level is considered not evaluable, this level will be excluded. All evaluable scores will be dichotomized into fusion and no fusion. To allow inclusion of patients with fusion of multiple segments, without increasing the influence of these patients on the outcome, each side in these patients will be statistically considered as one condition. This means that the possible outcomes in these patients are: no fusion (if none of the levels is fused), equal fusion (if an equal number of segments is fused per side) or fusion at one side vs. no-fusion at the other side in case one side contains more fused segments than the other side.

The basis for the primary efficacy analysis will be the test of non-inferiority by performing a McNemar's test. The null hypothesis is that inferiority in fusion rate of the MagnetOsTM Granules compared to the autograft condition is greater than 15% (PAG-PMAG \geq 15%); the alternative hypothesis is that the MagnetOsTM Granules fusion rate will not be lower than the autograft success rate by more than 15% (PAG-PMAG< 15%).

In addition to the McNemar's test, a generalized estimating equations (GEE) model will be used to examine fusion on each side of a vertebra, while accounting for clustering of vertebrae within patients. The outcome will be fusion (yes/no) and the explanatory variable will be treatment. Analysis will result in an odds ratio for MagnetOsTM Granules vs. autograft and a 95% confidence interval.

9.2 Primary safety analysis

(Serious) adverse events occurring during the study that may in any way be related to the surgical procedure will be reported in adverse event tables. The frequency and nature of events will be compared to events in control populations from literature.

9.3 Analysis of secondary outcomes

1. To compare the quantitative primary and secondary results of MagnetOs™ Granules to Attrax® Putty a McNemar's test will be used as well as the mentioned GEE model;
2. As mentioned for the primary analysis, the odds-ratios and 95% confidence intervals for fusion rates of MagnetOs™ Granules and autograft will be calculated by using the GEE model. This model also allows to assess the effect of an additional interbody fusion;
3. For the comparison of the fusion rate after two years, the same method as for the primary efficacy analysis will be used;
4. VAS pain scores will be analyzed using the paired t-test, or Wilcoxon signed rank test in case of not-normally distributed data. To investigate the effect of blinding on perceived donor site pain, pain scores reported by the patients of this study and the AxA study will be compared using the unpaired t-test or Mann-Whitney U test.
5. To investigate risk factors of both study populations regression analysis will be done.

9.4 Other study parameters

The following information will be reported and reviewed, but will not influence the comparisons between the treatment conditions as this is an intra-patient model.

- Demographics
- Nicotine use
- Pre-operative diagnosis
- Medical history
- Concurrent medical issues
- ASA classification
- Disease related medication use
- NSAID use
- Perioperative data
- Length of hospital stay
- EQ-5D-5L
- ODI
- Pain drawing
- NRS satisfaction

The clinical outcome measures, the EQ-5D-5L, ODI and VAS, will be analyzed with repeated measurements to assess changes over time. For continuous data, an independent t-test or Mann Whitney U test (depending on the distribution of the data) will be used to compare the

clinical outcomes of fused and non-fused patients. For categorical data, the Chi-square test will be used. A logistic regression analysis will be performed to analyze the effects of smoking and NSAID use on fusion status.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (version October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO), the Medical Device Directive 93/42/EEC and Standard Operating Procedures (SOPs) of the UMC Utrecht.

10.2 Recruitment and consent

Adult patients qualifying for instrumented posterolateral spinal fusion of one or multiple levels in the thoracolumbar and lumbosacral region (T10-S2) with the use of autograft will be recruited by their treating orthopaedic surgeon or his/her clinical assistant. If the patient meets the in- and exclusion criteria and is willing to receive further information, he/she will be informed about the study and its objectives by a member of the study team authorized by the local principal investigator, and will be asked to participate. In addition to the oral information, the patient will get a written information letter (see document E1) and at least one week to discuss the study with others (including an independent physician) and consider their decision. After obtaining a written informed consent (see document E2), the patient will be enrolled in the study and will get a unique study identification-number according to which all obtained data will be registered. Informed consent will be recorded in the electronic patient dossier. The original signed informed consent form must be retained in the Investigator Site File; the patient will receive a copy.

10.3 Objection by minors or incapacitated subjects

Minors or incapacitated/incompetent subjects will not be recruited or enrolled in this study.

10.4 Benefits and risks assessment, group relatedness

Enrolled patients have the benefit of shorter surgery time and maintenance of iliac bone stock due to reduced bone harvesting from the iliac crest. The risk for a functional adverse outcome due to non-union with MagnetOs™ Granules is minimal, as the contralateral side will always be treated in the conventional way and both sides of the spine are instrumented. If MagnetOs™ Granules appears to perform inferior to the autograft condition, a one-sided functional posterolateral spinal fusion as a result of iliac crest bone, together with bilateral rigid instrumentation, is likely to compensate for this failure.

10.5 Compensation for injury

The sponsor has a liability insurance which is in accordance with article 7, subsection 6 of the WMO (see document G2). All participating centers have their own liability insurance. This is laid down in the clinical trial agreements.

The sponsor also has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to all research subjects through injury or death caused by the study (see document G1). The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

Data will be handled confidentially according to the Dutch Personal Data Protection Act. After obtaining a written informed consent, the patient will get a unique study identification-number (consisting of a hospital ID and consecutive number) according to which all data obtained for this study will be registered. Informed consent will be recorded in the electronic patient dossier. The original signed informed consent forms will be retained in the Investigator Site File, stored in a locked closet or room. The key to the study identification-numbers will be safeguarded by the local principal investigators.

All study data will be collected in Castor EDC and exported to SPSS for data analyses. The local study teams can enter the data directly in the web-based eCRF or after completing a printed version of the eCRF. Patients will receive an email via Castor EDC with a personalized link to the digital questionnaires. If patients prefer paper questionnaires, they should be completed at the outpatient clinic. To be able to reproduce the study findings and to help future users to understand and reuse the data, all changes made to the raw data and all steps taken in the analyses will be documented in queries, syntaxes or text documents.

Only the local study team authorized by the local principal investigator, the monitor and Health and Youth Care Inspectorate have access to the source data in the medical patient dossier; the principal investigator and coordinating investigator will only receive the completed CRFs and corresponding images, as well as the (S)AE-forms and relevant additional information.

At the UMC Utrecht, research data and files will be stored and archived on the research network disc of the division of Surgical Specialties.

All study data will be saved for at least 15 years after the end of the study.

11.2 Monitoring and Quality Assurance

The study will be monitored by trained and independent monitors from TFS, according to the guidelines of the Dutch Federation of University Medical Centers (NFU), adjusted to the UMC Utrecht. The purposes of monitoring are to verify that:

1. The rights and well-being of human subjects are protected;
2. The reported trial data are accurate, complete, and verifiable from source documents;

3. The conduct of the trial follows the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement(s).

The potential risks associated with participation in this study can be classified as 'negligible risk'. This is because the material (tricalcium phosphate TCP) is biochemically well characterized and applied for more than 30 years in millions of patients worldwide. The difference of this material is the microstructure which is more porous and therefore more bone inductive. A very similar material was investigated in the recently performed AxA study (METC nr 13-192) where no material related adverse events were reported. The risk of performing less good than autologous bone is mitigated by the bilateral (intrapatient) design, where for each fusion level always one side is treated with autologous bone and the other side with MagnetOs™ Granules. In addition the fusion is always instrumented with posterior fixation which immobilizes the segment and will cause a fusion in the long term. Therefore the addition of Magnetos on one side is fundamentally different from a standalone application and should be regarded as an augmentation to enhance fusion on that side while traditional fusion is achieved with autograft on the other side and the rigid fixation. This negligible risk is compensated with the definite advantage of less autologous bone graft donor side morbidity.

The following on-site monitoring strategy (per site) is proposed:

1. One initiation visit;
2. Three monitor visits;
3. One close-out visit.

More details on the monitoring strategy are given in document K6a.

11.3 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

11.4 Annual progress report

The principal investigator/sponsor will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events, other problems, and amendments.

11.5 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the principal investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.6 Public disclosure and publication policy

Kuros' participation in funding will be disclosed to any audience to which any information or results of this study are presented. The local principal investigators are permitted to present at symposia, national or regional professional meetings, and to publish in journals, theses or dissertations, or otherwise of their own choosing. Kuros will be provided with a manuscript of any proposed publication at least forty-five (45) days prior to submission, and with the abstract of any proposed presentation at conferences at least thirty (30) days prior to submission, in order to review any such manuscript and to request changes therein to protect inventions and/or proprietary rights and proprietary interests. See the agreement for more detailed information on disclosures and the publication policy.

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

This study involves the application of a registered product (MagnetOs™ Granules) to be used within the indication and not in combination with other products.

12.2 Synthesis

As previously indicated, the main risk of this study will be inferior performance of MagnetOs™ Granules, resulting in a lower (unilateral) posterolateral fusion rate. Based on the preclinical studies as described in the Investigator's Brochure (see Chapter 6), this risk can be considered minimal. Furthermore, the most closely related and currently investigated MagnetOs™ Granules has shown to perform superior to currently widely applied competitor TCPs like Vitoss® and Actifuse®. To minimize risks, MagnetOs™ Granules will only be applied unilaterally in instrumented posterolateral spinal fusions. The contralateral side will receive autologous bone graft that is considered the gold standard for this surgical procedure. Furthermore, instrumentation will stabilize the spinal segments to allow an optimal environment for bony fusion. All patients may benefit from the study in terms of less surgery time and maintenance of iliac bone stock, since only half of the required bone graft will be harvested from the iliac crest. When MagnetOs™ Granules is indeed non-inferior to iliac crest bone graft, this harvesting procedure may be abandoned in future patient populations.

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APPENDIX A. STATISTICAL ANALYSIS

The statistical methods were designed previously for the AxA trial in collaboration with a medical statistician, dr. R.K. Stellato of the Julius Center, UMC Utrecht, the Netherlands.

Sample size calculation

The numeric results for a non-inferiority (one-sided) test of a difference are shown in the table below. Based on a 1:1 paired assignment of AttraX® Putty to the control condition with autograft, a power of 80%, a one-sided alpha of 0.05, a non-inferiority margin of 15%, an estimated fusion percentage of 50% for unilateral grafting and 70% concordance between both sides of the fusion trajectory, the required sample size for the primary outcome is 84 patients.

Definitions

- Power: probability of rejecting a false null hypothesis;
- N: number of subjects, sample size;
- De: maximum difference between the two proportions that is still called 'equivalent';
- Da: actual difference between Pt and Ps, i.e. Da = Pt-Ps;
- Pt: the response proportion to the treatment (experimental or new) test;
- Ps: the response proportion to the standard (reference or old) test;
- Nuisance Parameter: value that is needed, but is not a direct part of the hypothesis;
- Alpha: probability of rejecting a true null hypothesis;
- Beta: probability of accepting a false null hypothesis.

Power Analysis of Non-Inferiority Tests of Correlated Proportions

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Power	Sample	Equiv.	Actual	Treatment	Standard	Nuisance	Alpha	Beta
	Size (N)	Difference (De)	Difference (Da)	Proportion (Pt)	Proportion (Ps)	Parameter (P01+P10)		
130.10000-0.10000								
0.80005	5889	0.10000	-0.08000	0.42000	0.50000	0.30000	0.05000	0.19995
0.80003	1400	0.10000	-0.06000	0.44000	0.50000	0.30000	0.05000	0.19997
0.80024	591	0.10000	-0.04000	0.46000	0.50000	0.30000	0.05000	0.19976
0.80037	315	0.10000	-0.02000	0.48000	0.50000	0.30000	0.05000	0.19963
0.80135	191	0.10000	0.00000	0.50000	0.50000	0.30000	0.05000	0.19865
0.80000	24761	0.11000	-0.10000	0.40000	0.50000	0.30000	0.05000	0.20000
0.80009	2623	0.11000	-0.08000	0.42000	0.50000	0.30000	0.05000	0.19991
0.80024	899	0.11000	-0.06000	0.44000	0.50000	0.30000	0.05000	0.19976
0.80050	436	0.11000	-0.04000	0.46000	0.50000	0.30000	0.05000	0.19950
0.80053	250	0.11000	-0.02000	0.48000	0.50000	0.30000	0.05000	0.19947
0.80009	158	0.11000	0.00000	0.50000	0.50000	0.30000	0.05000	0.19991
0.80005	6202	0.12000	-0.10000	0.40000	0.50000	0.30000	0.05000	0.19995
0.80006	1479	0.12000	-0.08000	0.42000	0.50000	0.30000	0.05000	0.19994
0.80009	626	0.12000	-0.06000	0.44000	0.50000	0.30000	0.05000	0.19991
0.80044	335	0.12000	-0.04000	0.46000	0.50000	0.30000	0.05000	0.19956
0.80164	204	0.12000	-0.02000	0.48000	0.50000	0.30000	0.05000	0.19836
0.80170	134	0.12000	0.00000	0.50000	0.50000	0.30000	0.05000	0.19830
0.80009	2763	0.13000	-0.10000	0.40000	0.50000	0.30000	0.05000	0.19991
0.80032	950	0.13000	-0.08000	0.42000	0.50000	0.30000	0.05000	0.19968
0.80046	462	0.13000	-0.06000	0.44000	0.50000	0.30000	0.05000	0.19954
0.80079	266	0.13000	-0.04000	0.46000	0.50000	0.30000	0.05000	0.19921
0.80094	169	0.13000	-0.02000	0.48000	0.50000	0.30000	0.05000	0.19906
0.80255	115	0.13000	0.00000	0.50000	0.50000	0.30000	0.05000	0.19745
0.80000	1558	0.14000	-0.10000	0.40000	0.50000	0.30000	0.05000	0.20000
0.80039	662	0.14000	-0.08000	0.42000	0.50000	0.30000	0.05000	0.19961
0.80043	355	0.14000	-0.06000	0.44000	0.50000	0.30000	0.05000	0.19957
0.80021	216	0.14000	-0.04000	0.46000	0.50000	0.30000	0.05000	0.19979
0.80181	143	0.14000	-0.02000	0.48000	0.50000	0.30000	0.05000	0.19819
0.80338	96	0.14000	0.00000	0.50000	0.50000	0.30000	0.05000	0.19662
0.80029	1001	0.15000	-0.10000	0.40000	0.50000	0.30000	0.05000	0.19971
0.80033	488	0.15000	-0.08000	0.42000	0.50000	0.30000	0.05000	0.19967
0.80093	282	0.15000	-0.06000	0.44000	0.50000	0.30000	0.05000	0.19907
0.80163	180	0.15000	-0.04000	0.46000	0.50000	0.30000	0.05000	0.19837
0.80062	122	0.15000	-0.02000	0.48000	0.50000	0.30000	0.05000	0.19938
0.80098	84	0.15000	0.00000	0.50000	0.50000	0.30000	0.05000	0.19902
0.80005	697	0.16000	-0.10000	0.40000	0.50000	0.30000	0.05000	0.19995
0.80031	375	0.16000	-0.08000	0.42000	0.50000	0.30000	0.05000	0.19969
0.80039	229	0.16000	-0.06000	0.44000	0.50000	0.30000	0.05000	0.19961
0.80183	152	0.16000	-0.04000	0.46000	0.50000	0.30000	0.05000	0.19817
0.80168	106	0.16000	-0.02000	0.48000	0.50000	0.30000	0.05000	0.19832
0.80386	75	0.16000	0.00000	0.50000	0.50000	0.30000	0.05000	0.19614
0.80009	514	0.17000	-0.10000	0.40000	0.50000	0.30000	0.05000	0.19991
0.80095	298	0.17000	-0.08000	0.42000	0.50000	0.30000	0.05000	0.19905
0.80029	190	0.17000	-0.06000	0.44000	0.50000	0.30000	0.05000	0.19971
0.80159	130	0.17000	-0.04000	0.46000	0.50000	0.30000	0.05000	0.19841
0.80146	84	0.17000	-0.02000	0.48000	0.50000	0.30000	0.05000	0.19854
0.80249	66	0.17000	0.00000	0.50000	0.50000	0.30000	0.05000	0.19751

Primary analysis

The CT score will be dichotomized into fused and not fused. To allow the inclusion of patients with intended fusion of multiple segments, the sum of fusions on each side of the spine of these patients will be compared. If one side of a fusion-level is considered not evaluable, this will be excluded as well as the contralateral level. In multilevel patients, superiority (in number of fused levels) of one side, will be considered fusion and the other side non-fusion to prevent an unequal influence of patients with multi-level fusions. Although fusion assessment by this method gives more a "relative performance score" than a "fusion rate," analyzing the fusion rate of individual levels will not be adequate. There is strong reason to believe that the fusion rate will vary from patient to patient, so the fusion of individual vertebrae from one patient will not be independent. Since the study is randomized, this dependence should have no effect on a point estimate for the difference in fusion rates for MagnetOsTM Granules vs. autograft, but will certainly affect the standard error of the difference, thereby making standard statistical inference unreliable.

Although we know of no way of generating correct p-values or confidence intervals for differences in fusion rates using clustered data, it is possible to use a multi-level model (in which the clustering of vertebrae within patients is taken into account) to examine the effect of MagnetOsTM Granules and autograft on fusion, and using the vertebrae as the primary level of analysis. Such a model will produce an odds ratio instead of a difference in fusion rates. Since a sample size calculation based on a multi-level analysis requires a number of assumptions that cannot be supported by current data, we decided to perform sample size calculation based on the McNemar's test, and to use the multi-level model as a secondary analysis since it will make better use of the complete data while correctly taking the clustering into account.

Secondary Analysis

A three-level generalized estimating equations (GEE) model will be used to examine fusion on each side of a vertebra, while accounting for clustering of measurements (fusion on each side of the spine) within spinal levels and within patients. The GEE model will use compound symmetry working correlation matrices for correlation of measurements within vertebrae and vertebrae within patients. In this model, the presence of interbody fusion can also be incorporated as a separate factor. The outcome will be fusion (yes/no) and the explanatory variable will be the treatment condition. Analysis will result in an odds ratio for MagnetOsTM Granules vs. autograft and a 95% confidence interval.

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APPENDIX B. SUGGESTED SURGICAL TECHNIQUE

Step 1: Patient positioning

The patient is placed in the prone position. To avoid abdominal compression, separate rolls or specific frames can be used. Preferably a foil skin drape is used.

Step 2: Exposure

After sterile draping, a single midline incision centered on the intended fusion area is made in both the skin and the fascia. The paraspinal muscles are subperiosteally stripped from the spinous processes and retracted laterally. The facet joints and transverse processes are exposed.

Step 3: Instrumentation

After partial removal of the involved facet joints, the surgeon determines the exact surgical procedure concerning the insertion of the instrumentation. Depending on the surgeon's preference, pedicle screws can be inserted now or after decompression. The rod fixation can be inserted at this time or after placement of the graft.

Step 4: Decompression

If necessary, a decompression of the posterior spine is performed according the surgeon's preference. The decompression may involve a whole or partial laminectomy and may include one or more levels.

Step 5: Decortication

The next step is meticulous and thorough decortication of all of the available cortical surfaces, including the dorsal, superior and inferior surfaces of the transverse processes. It may be necessary to extend the longitudinal incision to get good exposure! In addition, the dorsal and lateral aspects of the lamina and pars interarticularis, from the most proximal vertebra to the most distal vertebra to be included in the fusion should be denuded. If the facet joints remain, these should be prepared for fusion by removing the exposed articular surfaces.

Step 6: Graft preparation and volume measurement

All bone obtained from decompression and decortication is stripped from soft tissue and morcellized to 2-4mm pieces. The volume is assessed by slight compression in a 20mL syringe.

Step X: Additional interbody fusion procedure

According to the surgeon's preference, an interbody cage can be used (PLIF or TLIF). This cage is preferably filled with local autograft bone and positioned before disclosure of the randomized allocation. If the surgeon prefers iliac graft bone, both sides of the spine have to be prepared for posterolateral bone grafting before the allocated side of the autograft (and bone graft harvesting) is disclosed.

Step 7: Disclosure of the random allocation

After instrumentation and preparation of the fusion trajectory the envelope containing the allocation side of the autograft and MagnetOs condition and thereby the allocation of the iliac harvesting side, is opened.

Step 8: Harvesting of iliac crest autograft

Only one side of the pelvis can be used for iliac crest bone harvesting. This must be the same side as allocated to the autograft posterolateral spine fusion condition. The surgeon can use any technique that exposes the iliac crest to obtain corticocancellous bone, but preferably no additional skin incision is made and harvesting through a posterior cortical window is advised. A total autograft volume of 8-10cc per side and fusion level is intended. The contribution of iliac bone graft to this volume has to be at least 50%. For example in a L3-S1 three-level fusion with 10cc of local bone, another 20cc of bone from the iliac crest is required to reach 3x10cc. If local bone is 20cc, 5cc of this will not be used as local bone can only be 15cc (= 50% of 30cc). If the 10cc per level and side cannot be reached because of limited iliac bone graft, the autograft volume has to be extended with local bone. In case the volume remains below 8cc per side, this is accepted as a consequence of autologous bone grafting. The MagnetOs condition will always be 8-10cc and not combined with local bone.

Step 9: Placement of the graft

Both grafts, the autograft and MagnetOsTM granules, are placed on the allocated side around the instrumentation and in the decorticated lateral gutters, bridging the dorsal surfaces of the transverse processes, facets and laminae. No graft is placed on the dura. No additional substances like blood or bone marrow are added. If not yet done at step 3, the rod fixation is inserted at this time.

Step 10: Patient closing

The patient is closed using the technique preferred by the surgeon. If vancomycin or another antiseptic procedure is used, this should be recorded on the surgical form of the CRF.

APPENDIX C. CT ASSESSMENT

Aim

To compare the fusion rate of the MagnetOsTM granules side to the autograft side after 1 and 2 years, by using CT-scans.

Method primary analysis

For the primary analysis, all included patients will be CT-scanned at the treating center. The CT-scan will be scheduled at 12-15 months after surgery. The standard local protocol for the spine will be used when this provides a slice thickness of ≤ 1 mm. Otherwise, the slice thickness will be adapted. To allow all desired planar reconstructions, the data will be captured and stored as volume scans. The raw volume dataset (DICOM scans), and preferably reconstructions in the coronal, sagittal and axial, will be de-identified and stored on a CD-ROM and send to the principal investigator for assessment by a panel consisting of at least one spine surgeon and an uninvolved specialist. Based on literature review and experience with the AxA trial we developed the following protocol.

Stepwise protocol

- All patients will be scanned with a volume scan with ≤ 1 mm slice thickness;
- Data will be captured and stored as volume scans;
- All scans will be assessed independently by at least two observers, blinded to the treatment allocation;
- First, the fusion level and side will be identified and assessed in three planes: coronal, sagittal and axial;
- Osteotomized segments will be excluded from the analyses. If one side of a fusion-level is considered not evaluable, this level will be excluded as well;
- If obvious indications of mobility like material failure or apparent pseudoarthrosis exist, the level will be considered a non-union;
- If not, the presence of an obvious bone bridge/fusion between the posterolateral vertebra will be determined;
- Fusion is scored based on assessment in three planes and at two locations, to indicate the probability that the bony bridge is graft related:
 - Likely graft related is a bony bridge between the transverse process and >1 mm lateral or above the rod;
 - Probably not graft related is a bone bridge directly around the rod, between the lamina or fusion of the facet joints;

- An obvious fusion at any of these locations in each plane is scored 2 points, doubtful fusion is 1 point and non-union is 0 points;
- If the segment is fused in at least one plane (2 points) and does not show obvious absence of fusion in both the other planes (scores at least 1 in at least one of the other planes) it is regarded as fused;
- The points of the graft related and non-graft related locations cannot be accumulated;
- Finally, the presence of an interbody fusion as a result of an interbody fusion device will be determined in two planes and scored similarly (fusion is at least 3 points).

In case of disagreement on overall fusion between the observers, a third investigator will be counseled to get consensus. If disagreement persists, the fusion will be scored as doubtful.

Method secondary analysis

For the other secondary analyses, the 2 year CT-scans will be assessed similarly.