Relation between radiological assessment and biomechanical stability of lumbar interbody fusion in a large animal model

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Abstract

Purpose To relate the progress of vertebral segmental stability after interbody fusion surgery with radiological assessment of spinal fusion.

Methods Twenty goats received double-level interbody fusion and were followed for a period of 3, 6 and 12 months. After killing, interbody fusion was assessed radiographically by two independent observers. Subsequently, the lumbar spines were subjected to four-point bending and rotational deformation, assessed with an optoelectronic 3D movement registration system. In addition, four caprine lumbar spines were analysed in both the

native situation and after the insertion of a cage device, as to mimic the direct post-surgical situation. The range of motion (ROM) in flexion/extension, lateral bending and axial rotation was analysed ex vivo using a multi-segment testing system.

Results Significant reduction in ROM in the operated segments was already achieved with moderate bone ingrowth in flexion/extension (71 % reduction in ROM) and with only limited bone ingrowth in lateral bending (71 % reduction in ROM) compared to the post-surgical situation. The presence of a sentinel sign always resulted in a stable vertebral segment in both flexion/extension and lateral bending. For axial rotation, the ROM was already limited in both native and cage inserted situations, resulting in non-significant differences for all radiographic scores.

Discussion In vivo vertebral segment stability, defined as a significant reduction in ROM, is achieved in an early stage of spinal fusion, well before a radiological bony fusion between the vertebrae can be observed. Therefore, plain radiography underestimates vertebral segment stability.

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Keywords Lumbar spinal fusion · Animal model · Motion-segment stability · Mechanical testing · Radiography

Introduction

Lumbar interbody fusion is performed to relieve the clinical symptoms associated with symptomatic degenerative changes in the intervertebral disc. This is achieved by stabilizing and realigning the spine and by restoration of the intervertebral disc height. The interbody spacer (cage) can be used as an adjunct to posterior pedicle screw

instrumentation or as a stand-alone anterior fusion cage [16]. The surgical goal in spinal fusion surgery is the establishment of a bony fusion between adjacent vertebrae that results in a stable fixation of two adjacent vertebral bodies. The various techniques to obtain this bony fusion using either stand-alone cages, or cages with additional instrumentation, have been analysed extensively biomechanically in vitro [4, 8, 19, 27]. These experimental setups actually investigate the direct post-surgical stability of the vertebral motion segment, and conclusions mainly relate to the first day after surgery [1]. In contrast, the end-stage of spinal fusion surgery is investigated in in vivo studies, using various types of materials and techniques, with or without the addition of growth factors, to assess the occurrence of a bony fusion between adjacent vertebrae. Although the pursued solid fusion should result in little or no motion between the vertebrae [17], it is unknown how vertebral segment stability develops from surgery to solid fusion [6, 16]. In clinical practice, the need to prove solid fusion has been doubted by Brantigan, stating that “when a successful clinical result (i.e.: no pain) is obtained, it is probably unnecessary to radiologically prove bony arthrodesis in these patients [17]”. It further can be considered that radiographical fusion implies mineralization of the cartilaginous matrix, while well before that an unmineralized matrix of cartilaginous tissue has already been formed, possibly enhancing the biomechanical stability. Therefore, we hypothesized that substantial vertebral segment stability is already achieved prior to radiographically proven solid arthrodesis in our spinal fusion goat model.

In this goat interbody fusion study ($N = 20$) we evaluated the biomechanical and radiological fusion state of 20 caprine lumbar spines, following double-level fusion procedures ($N = 40$ vertebral segments) after 3, 6 and 12 months. Ex vivo segment stability was related to the stage of bone ingrowth as assessed by radiography. The current study does not differentiate between follow-up periods, but rather distinguishes groups based on their radiographic score (RS). As a control, four caprine lumbar spines were analysed in both the native situation and after the insertion of a cage device, to mimic the direct post-surgical situation.

Materials and methods

Animals, surgical technique and specimen preparation

Surgical procedure and animal care were performed in compliance with the regulations of the Dutch legislation for animal research and the Animal Ethics Committee of the VU University Medical Center approved the protocol. Twenty skeletally mature female Dutch milk goats

(60–80 kg) were used for this biomechanical study. From these, eight spines were harvested 3 months after surgery and nine spines 6 months post-surgery. Three goats were killed after 12 months. As controls, the lumbar spines of four goats, killed for an unrelated experiment, were collected.

Surgery was performed under general anaesthesia with endotracheal intubation. Prophylactic antibiotic treatment was administered once before surgery. Prior to the procedure, standard lateral radiographs were taken to detect the presence of spontaneous disc degeneration.

Interbody cages were designed equivalent to the cages described earlier [13, 23]. The cages were made of carbon-reinforced polyetheretherketone (PEEK) with a vertical and rectangular geometry ($10 \times 10 \times 18$ mm, wall thickness 1.5 mm). The cage filler composed of either tri-cortical autologous bone grafted from the left iliac crest, or a degradable filler material [10, 14, 25].

The surgical procedure has previously been described in detail [13, 23, 24]. Briefly, L1–L2 and L3–L4 were identified and approached via a left retroperitoneal approach using fluoroscopy. The intervertebral discs (IVD) of interest were exposed after mobilization of the psoas muscle. Under fluoroscopic guidance, a 2-mm guidewire was centred transversely in the IVD. Along this guidewire, a hole was drilled through the IVD and the adjacent endplates with an 8-mm drill bit. A 10×10 mm box gauge was placed over the drill and used to punch a transverse square defect, in which a cage could be inserted. The caprine lumbar IVD are about half the size of a human disc [12, 22], resulting in a disc height of 4–5 mm [22]. Using a 10×10 mm box gauge both endplates are removed and trabecular bone can be seen during surgery. With a trabecular bone density about twice that in humans, it can withstand axial compression with the endplates opened [22], in contrast to the human situation, in which the endplates are therefore carefully prepared.

After 1 week, when the surgical wound was healed, the animals were moved to a large in- and outdoor environment without restrictions. Health status, eating habits, and ambulatory activities were monitored daily. At the designated time, the goats were sedated with 10 mg/kg ketamine intramuscularly. Then the goats were euthanized with an intravenous overdose of sodium pentobarbital (20 mg/kg), after which gross pathological examination was performed and the lumbar spines were harvested. Immediately after killing, the lumbar spines were excised above Th13 level and below L5, resulting in a spinal segment of six vertebrae. Subsequently, the spines were trimmed of residual musculature, while leaving all ligamentous tissue intact, wrapped in phosphate buffered saline (PBS) soaked towels and stored at 4 °C. Within 24 h, four L-shaped markers were rigidly fixed in the ventral–midsagittal plane on the

four vertebral bodies of L1, L2, L3 and L4 (Fig. 1). Each marker was equipped with three light-emitting diodes (LEDs) for optoelectronic 3D movement registration. The cranial (T13) and the caudal (L5) spine ends were completely embedded in two metal cups, filled with cerrow-low147 (Cerro Metal Products Co., Bellefonte, PA) heated at 60 °C. The IVD of T13–L1 and L4–L5 were not embedded and therefore allowed full movement. During testing, both operated segments (L1–L2 and L3–L4) and the interadjacent segment (L2–L3) were analysed simultaneously. After cooling and hardening of the alloy, the specimen was mounted on a four-point bending device (Fig. 1). During the preparation and testing procedure, the specimens were kept moist by spraying saline solution (NaCl 0.9 %).

Radiological and biomechanical assessment

At the designated end-points of 3, 6 and 12 months, standard lateral radiographs of the lumbar spine of each goat were taken. The first lateral radiograph was taken just prior to euthanization to preserve muscle tonus. A second radiograph was taken of the excised lumbar spine to reduce soft tissue obscurement. Finally, after biomechanical testing, a third lateral radiograph was taken of paramidsagittal

slices of each operated motion segment (see below for details).

Biomechanical testing was performed as described earlier [3] using a custom made four-point bending device in which flexion/extension, right and left lateral bending, and right and left axial rotation could be applied. This device was driven by a Zwick mechanical material testing system (Zwick Roell, Ulm, Germany), mounted on a hydraulic mechanical testing device (Instron 8872, Canton MA, USA). The lumbar spine was positioned in its neutral, horizontal position where the load was set to zero. Subsequently, moments of 3.0 Nm were gradually applied in the tested direction, with a rotation speed of 1.0°/s. For axial rotation, the maximum applied moment was 2.0 Nm. Specimens were tested for ten continuous cycles and the average data of all cycles were analysed. Motions of the LEDs were recorded by an optoelectronic 3D movement registration system with an array of three cameras (Optotrak 3020, Northern Digital Inc, Waterloo ON) (see video 1). Before testing, the axes of the Optotrak system were aligned with the anatomic axes of the spinal segment. The three-dimensional resolution for this system at 2 m distance is 0.01 mm [3]. The torque applied to the upper level of the chain of vertebral segments and resisted by the lower level results in an equal torsional/bending moment for all levels; however, the deformity will vary between the levels as a function of fusion.

For the control spines, the first biomechanical test comprised the native situation. Subsequently, to gain insight in the biomechanical situation directly post-operative, biomechanical testing was performed after the insertion of an interbody spacer (cage) at the L1–L2 and L3–L4 level, identical to the surgical *in vivo* procedure received by the experimental spines (see above). Both biomechanical tests were identical to the biomechanical test described above for the explanted spines.

A customized version of a previously written program (Matlab; Mathworks, Natick MA, USA) was used for data analysis [3]. Movement in the main intended direction (flexion/extension, lateral bending or axial rotation) was plotted as a function of load to obtain moment–angular displacement curves. The range of motion (ROM) of each motion segment was calculated as the maximum angular motion (degrees) between the maximum moments in positive (+3 Nm) and negative directions (−3 Nm).

After biomechanical testing, the operated motion segments were excised and 4-mm thick paramidsagittal sections were made with a water-cooled band saw (EXAKT, Norderstedt, Germany). Lateral radiographs of these specimens were used to estimate interbody fusion, based on a validated radiographic score [24] which was modified for this study as follows. The rectangular cage on the radiograph was divided in three equal parts along the spinal axis

Fig. 1 An explanted lumbar spine is placed in a custom made four-point bending device using metal filled cups with cerrow-low147 on both ends. The spine is mounted with L-shaped markers, containing each 3 LEDs for 3D movement registration. The vertebral bodies are indicated and the bridging intervertebral discs can be seen, with interbody spacers inserted at the L1–L2 and L3–L4 levels

(Fig. 2). When no bone ingrowth was observed, the segment was scored as RS 0. For RS 1, bone ingrowth was present in the outer one-third of the cage. When bone had grown into the middle third of the cage, the specimen was qualified as RS 2. RS 3 was achieved when bony contact was present throughout the sagittal plane of the cage. The presence of a ventral bony fusion (sentinel sign) was always scored as RS 4, irrespective of the bone growth inside the cage.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS Inc, Chicago USA). Comparing the different radiographic scores and their ROM was done using a univariate general linear model and Tukey's post hoc test. To test the differences between the native segments before and after cage insertion, a Wilcoxon matched-pairs signed-ranks test was used. Correlations were analysed using Spearman's rank two-tailed correlation coefficients with the following interpretation; 0.5–0.75 indicates good relationship [5] and >0.75 indicates excellent relationship [5].

Fig. 2 Accompanied by actual radiographs, a schematic lateral diagram in which the rectangular interbody spacer is divided into three equal parts to obtain a radiographic score (RS) ranging from 0 (no bone ingrowth) to 4 (ventral bony fusion) is depicted. The presence of bone in each or both of the outer thirds of the cage, resulted in a RS 1 (1). Bone present in the middle third of the cage but without bridging contact was indicated RS 2 (2), and RS 3 was scored when a bony fusion inside the cage was present (3). With the presence of a ventral bony fusion, the RS score of 4 was indicated irrespective of the bone formation inside the interbody spacer (4)

The interobserver agreement for the radiographic score was calculated using Cohen's kappa value. Differences were considered statistically significant when $p < 0.05$.

Results

One goat in the 12-month follow-up group was preliminary euthanized after 9 months due to severe pneumonia and excluded from the study. The remaining goats included in this study recovered uneventfully from the surgical procedure and no complications were observed during follow-up. Normal ambulatory activities were regained on the first postoperative day. The lateral radiographs taken prior to initial surgery showed no signs of spontaneous disc degeneration.

At the time of killing, the L1–L2 segment of one single goat in the 12-month follow-up group had an infection inside the interbody spacer and was therefore left out of the biomechanical analysis. Due to insufficient fixation of an optoelectronic marker, the analysis of one segment with RS 2 was excluded from the flexion/extension test. Two segments (one RS 2 and one RS 4) of the same spine were excluded from analysis due to improper embedding during lateral bending. In the control group, the axial rotation analysis of one segment was excluded for the same reason.

Radiological and biomechanical assessment

The lateral radiographs taken just prior to euthanization could not be used to determine the radiographic score due to overlapping soft tissue contours. In most cases, the radiographs taken of the excised lumbar spines were also not suited for analysis due to obscuring transverse processes. Both radiographs were therefore left out of the analysis. In accordance with van Dijk et al. [24], the lateral radiographs of the paramidsagittal sections were scored by two observers (RJK and MH). The kappa value concerning the inter-observer reliability of this modified RS score was 0.82. In the event of a different score, both observers discussed the radiograph and assigned the appropriate score to the corresponding segment. From the remaining 37 segments, two segments scored RS 0. Both RS 1 and RS 2 were scored eight times, while RS 3 was assigned to seven segments. Finally, 12 segments had a sentinel sign and were scored RS 4. Since only two segments had RS 0, these were left out of the statistical analysis, but they will be discussed below.

Both the biomechanical and radiographic data of the 3, 6 and 12 months group were combined in the evaluation, to analyse various stages of radiographic consolidation of the operated segments. Then, comparison of the ROM for all three directional planes could be made. All ROM values,

◀ **Fig. 3** Schematic representation of the range of motion (ROM) for the RS scores 1–4 and the native segments, before and after cage insertion. **a** Range of motion for flexion/extension. Significant lower values ($p < 0.05$ for RS 2 and 3, $p < 0.001$ for RS 4) are indicated by either **a** compared to native, or **b** compared to cage insertion, (**b**) for lateral bending. Significantly different values are indicated by either **a** compared to native, or **b** compared to cage insertion ($p < 0.001$, except for cage insertion compared with native $p < 0.05$), and **c** for axial rotation in which significantly lower values ($p < 0.01$) are indicated by **c** compared to RS 1. Values are represented as mean $\pm$ SD

and the reduction in ROM with advancing consolidation compared to the post-surgical situation (cage insertion) are shown in Tables 1 and 2.

In the control spines, the mechanical properties of three motion segments in each lumbar native goat spine were analysed before and after a double-level cage insertion. For flexion/extension, the ROM in the native situation was $3.21 \pm 1.83^\circ$ (mean $\pm$ SD) and stayed unaltered ($p = 0.99$) after cage insertion (Fig. 3a). With lateral bending, the ROM was $6.79 \pm 2.64^\circ$ for the native segments and increased significantly with 39 % after cage insertion (Fig. 3b). Axial rotation was not affected by cage insertion (Fig. 3c).

In the operated segments, the ROM decreased significantly from both the native and cage inserted situation when RS 2 (Table 1) or higher was obtained (Fig. 3a) for flexion/extension. The reduction in ROM compared to the post-surgical situation was 72 % (Table 2). No significant differences could be detected between the various RS groups, although RS 4 had the greatest reduction in motion (86 %; Table 2).

A significant decrease in ROM for lateral bending was already established at RS 1 (Table 1), compared to both the native and cage inserted situation (Fig. 3b), resulting in a 71 % reduction in motion (Table 2). Again, no significant differences were found between the different RS groups, but similar to flexion/extension, RS 4 had the greatest reduction in motion at 95 % (Table 2).

For axial rotation, all measured values were below 1° (Table 1), with the highest ROM in the RS 1 group. No significant differences were found between the native and cage inserted situation compared to the radiographic scores. However, a significant decrease in ROM between the RS groups was found for RS 4 when compared to RS 1 (Fig. 3c).

Comparing the data for the interjacent level (L2–L3) between the native and the post-surgical situation, no significant differences were found, with values for flexion/extension of $3.26 \pm 2.00^\circ$ for the native situation compared with 2.35 ± 1.53 for the post-surgical situation ($p = 0.34$). For lateral bending native versus the post-

Table 1 Overview of the range of motion related to the RS score

	Flexion/extension (°)	Lateral bending (°)	Axial rotation (°)
Native	3.21 ± 1.83	6.79 ± 2.64	0.38 ± 0.3
Cage insertion	3.20 ± 2.25	9.42 ± 4.13	0.44 ± 0.37
RS 0	0.95 (1.13, 0.75)	1.56 (1.54, 1.57)	0.56 (0.82, 0.31)
RS 1	2.03 ± 1.11	2.73 ± 1.14	0.74 ± 0.53
RS 2	0.91 ± 0.64	1.24 ± 0.79	0.41 ± 0.38
RS 3	0.79 ± 0.28	0.83 ± 0.83	0.22 ± 0.11
RS 4	0.44 ± 0.48	0.45 ± 0.39	0.16 ± 0.11

Data are presented as mean ± SD, except for RS 0 where the mean is given with the two original values in parentheses

Table 2 Overview of the reduction in range of motion with RS score compared to the post-surgical situation after cage insertion

	Flexion/extension ROM reduction (%)	Lateral bending ROM reduction (%)
Cage insertion	–	–
RS 0 (<i>N</i> = 2)	70	83
RS 1 (<i>N</i> = 8)	37	71
RS 2 (<i>N</i> = 8)	71	87
RS 3 (<i>N</i> = 7)	72	91
RS 4 (<i>N</i> = 12)	86	95

surgical situation was $6.48 \pm 1.84^\circ$ versus $5.58 \pm 2.94^\circ$, respectively, ($p = 0.31$). With axial rotation values of $0.27 \pm 0.11^\circ$ and $0.36 \pm 0.15^\circ$ for the native and post-surgical situation, respectively, again no significant differences were observed ($p = 0.36$).

To analyse whether the ROM decreases simultaneously for all three directional planes, their correlation coefficients were also calculated. The correlation coefficient between flexion/extension and lateral bending resulted in a good to excellent agreement ($r = 0.75$; Fig. 4a) [5]. A value of $r = 0.66$ was found between the flexion/extension and the axial rotation, also indicating a good agreement (Fig. 4b). Furthermore, a correlation coefficient of $r = 0.85$ (indicating excellent agreement [5]) was found between the ROM of lateral bending and the ROM of axial rotation (Fig. 4c).

To correlate the radiographic score to the corresponding ROM, correlation coefficients were computed. A good relationship was computed when correlating RS to flexion/extension ($r = 0.59$) and to axial rotation ($r = 0.70$). For lateral bending, an excellent relationship was found when correlated to the radiographic score ($r = 0.75$).

Discussion

The main aim of this study was to relate the progress of vertebral segmental stability after interbody fusion surgery

with the radiological assessment towards spinal fusion. Since no literature is present about this specific topic, this is, to our best knowledge, the first paper to describe a biomechanical analysis of advancing vertebral fusion as seen on plain radiography in a large animal model. Using a large animal model and an ex vivo multi-segment testing system, we confirmed the hypothesis that with advancing radiological fusion the vertebral segment motion decreases. However, a significant decrease in ROM for the operated vertebral segments already occurred in an early phase of radiological fusion (RS 2 (moderate ingrowth) for flexion/extension and RS 1 (limited ingrowth) for lateral bending).

Animal model and biomechanical assessment of the control spines

We performed a double-level fusion study in 20 goats using radiolucent stand-alone cages in the L1–L2 and L3–L4 segments. To approximate the human spine, we used a large animal model (goat), since previous studies in our group have shown similarities between the goat disc geometry, load, and morphology and the human disc [21]. Despite the fact that a goat is a quadruped, theoretical considerations show that the spine of the goat is loaded along its long axis, like humans [21]. However, differences in our goat model compared to the human situation include the smaller dimensions of the disc, the increased density of trabecular bone, the opening of the endplates during surgery and finally, the remaining disc tissue surrounding the cage after surgery.

To obtain baseline biomechanical characteristics of both native goat discs and the direct post-surgical situation, four control caprine lumbar spines were tested. After testing the native spines, ex vivo cage insertion was performed and subsequently tested. These tests showed that lumbar goat discs hardly allow for axial rotation, but mostly allow lateral bending. Furthermore, the paired testing of the control spines before and after interbody cage insertion showed an increased instability with lateral bending after the (lateral) insertion of the cage, which was likely due to

Fig. 4 Scatterplot diagrams representing the range of motion values for all directional planes with correlation between **a** flexion/extension and lateral bending $r = 0.75$ ($p < 0.001$), **b** flexion/extension and axial rotation of $r = 0.66$ ($p < 0.001$) and **c** lateral bending and axial rotation $r = 0.85$ ($p < 0.001$)

the unilateral destruction of the annulus fibres. This corroborates with previous single motion-segment experiments in our group [12, 24]. Clearly, in our goat spinal fusion model an increased instability after stand-alone interbody fusion surgery occurs. In the clinical setting, the ROM after insertion of interbody spacers has been reported to either decrease [2, 18, 20, 26], increase [11, 18] or remain unaltered [7], making a comparison to the human situation with respect to the direct post-surgical situation difficult. Despite this increased motion in lateral bending after device insertion in the goat model, we do usually obtain solid fusion in this goat stand-alone spinal fusion model in approximately 80 % of the cases [23].

Radiographic score and biomechanical assessment of the operated spines

This study was performed to gain insight into the biomechanical vertebral stability during the process of advancing fusion. For the assessment of interbody fusion, we sought a simple and cheap technique and therefore plain radiography was used. While the best method for assessing spinal fusion remains an ongoing debate [17], surgical exploration is assumed to be the golden standard. Unfortunately other reliable and non-invasive methods are not recognized [15]. Traditionally, spinal fusion was, and often still is, assessed using plain radiographs, but often without the use of explicit criteria. Some authors are convinced that not the radiograph itself, but the lack of movement seen on the radiographs is a more relevant measure of fusion [17].

An existing three-stage radiographic score [24] was modified for this study to assess for multiple stages of advancing fusion. We extended this score to obtain a five-stage scoring system, in which the presence of a ventral bony fusion (sentinel sign) resulted in the highest score (RS 4), irrespective of the stage of fusion inside the cage. All segments were scored by two observers and this resulted in an acceptable interobserver reliability ($\kappa = 0.82$). This radiographic score appeared inversely correlated to the ROM for flexion/extension, lateral bending and axial rotation. However, a discrepancy was found for RS 0 with average lower values for all three directions than the RS 1 values. This might be a coincidence due to the low number of RS 0 scored segments ($N = 2$) or it might possibly be related to a previously reported phenomenon by McAfee [16], who described a similar situation in a dog spinal fusion study in which the non-fused segments had a higher stiffness. He attributed this to the larger cross-sectional area of hypertrophic pseudarthrosis compared to the cross-sectional area of the fused segments [16], corroborating with our macroscopic observation of the relevant motion segments. The good to excellent relationships between

radiography and biomechanical testing for the radiographic scores 1–4 indicate the validity of the score from a biomechanical point of view.

Another interesting phenomenon is the presence of adjacent segment disease in which degeneration of the motion segment develops above or below another fused segment. In this study, no ROM differences between the native L2–L3 segment and the untreated L2–L3 segment could be detected. These results confirm earlier histological findings in our group that interjacent segments do not show degenerative signs following interbody fusion in our goat model [9].

In this study, we did not use spinal instrumentation, but significant reduction in ROM was already observed early in the process of interbody fusion, despite an initial increase in lateral bending motion after surgery. Clearly, the higher the radiographic score, the greater the reduction in ROM with the highest reduction in all directional planes with the presence of a sentinel sign (RS 4). Taken together, the presence of a sentinel sign would be the most stable situation following spinal interbody fusion, corroborating with previous literature [17]. Finally, other success criteria other than bony bridging should be developed, which better reflect the biomechanical effects of spinal fusion.

In summary, this lumbar fusion study in a large animal model showed that vertebral stability occurs well before solid arthrodesis can be seen on plain radiography.

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Conflict of interest None.

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