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Clinical Experiences of Non-fusion Dynamic Stabilization Surgery for Adjacent Segmental Pathology after Lumbar Fusion

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Abstract

Background
As an alternative to spinal fusion, non-fusion dynamic stabilization surgery has been developed, showing good clinical outcomes. In the present study, we introduce our surgical series, which involves non-fusion dynamic stabilization surgery for adjacent segment pathology (ASP) after lumbar fusion surgery.

Methods
Fifteen patients (13 female and 2 male, mean age of 62.1 years) who underwent dynamic stabilization surgery for symptomatic ASP were included and medical records, magnetic resonance images (MRI), and plain radiographs were retrospectively evaluated.

Results
Twelve of the 15 patients had the fusion segment at L4-5, and the most common segment affected by ASP was L3-4. The time interval between prior fusion and later non-fusion surgery was mean 67.0 months. The Visual Analog Scale and Oswestry Disability Index showed values of 7.4 and 58.5% before the non-fusion surgery and these values respectively declined to 4.2 and 41.3% postoperatively at 36 months (p=0.027 and p=0.018, respectively). During the mean 44.8 months of follow-up, medication of analgesics was also significantly reduced. The MRI grade for disc and central stenosis identified significant degeneration at L3-4, and similar disc degeneration from lateral radiographs was determined at L3-4 between before the prior fusion surgery and the later non-fusion surgery. After the non-fusion surgery, the L3-4 segment and the proximal segment of L2-3 were preserved in the disc, stenosis and facet joint whereas L1-2 showed disc degeneration on the last MRI (p=0.032). Five instances of radiologic ASP were identified, showing characteristic disc-space narrowing at the proximal segments of L1-2 and L2-3. However, no patient underwent additional surgery for ASP after non-fusion dynamic stabilization surgery.

Conclusion
The proposed non-fusion dynamic stabilization system could be an effective surgical treatment for elderly patients with symptomatic ASP after lumbar fusion.

Introduction
Since spinal fusion was introduced 100 years ago for the treatment of Pott’s disease, fusion technologies have dramatically advanced, and indications for fusion surgery have expanded to the areas of the cervical, thoracic, and lumbar spine.1 Fusion rates have exceeded 95% with the recent developments in fusion techniques; however, a large discrepancy is noted between radiologically successful fusion and clinical success rates. As an undesirable effect of fusion, adjacent segment pathology (ASP) has been well described. Referred formerly adjacent segment degeneration and adjacent segment disease (ASD) as well as adjacent segment pathology (ASP) were recently proposed as terms with which to refer to clinical and/or radiological changes at adjacent segments subsequent to a previous spinal surgery.2 The incidence and reoperation rates of ASP after fusion surgery have ranged from 25% to 35% within a 10-year period.
Due to the concern over the longevity of fusion constructs and the relatively high development of ASP, alternatives to spinal fusion have been developed with motion-preservation technology.

In 1994, Dubois et al. introduced the first pedicle-based dynamic stabilization system, Dynesys (Zimmer Spine, Minneapolis, USA). Several studies report that this system is an effective option in the treatment of lumbar degenerative disc disease, and indications for this system are expended with reference to spinal stenosis, spondylolisthesis and degenerative lumbar scoliosis. Currently, various types of dynamic stabilization systems are available.

To date, there is no research on the application of a non-fusion pedicle-based dynamic stabilization system for ASP after lumbar fusion surgery. With regard to this application, degenerative changes between an earlier fusion surgery and a later non-fusion dynamic stabilization surgery and degeneration after non-fusion surgery should all be assessed. Hence, this study compared degenerative changes arising on the intervertebral disc, central canal, and facet joint in cases of prior fusion surgery, prior non-fusion surgery, and upon the last follow-up. The clinical outcomes and radiologic ASP findings were evaluated to determine the effectiveness of the application of non-fusion surgery for ASP.

### Material and methods

#### Patient population

After the non-fusion dynamic stabilization system was procured by our institute in 2003, 108 patients underwent this type of surgery by a single surgeon as of the end of 2011. Of them, fifteen patients with symptomatic aggravation and radiologic confirmation of ASP after previous lumbar fusion surgery who had undergone the non-fusion dynamic stabilization surgery were included in the present study. Symptomatic patients with pseudarthrosis after fusion surgery, severe osteoporosis under medical treatment (≥ 2.5 T-score on the DEXA bone densitometry) were excluded.

#### Description of the dynamic stabilization system

Two types of pedicle-based dynamic stabilization systems were applied: the Dynesys system and the NFlex system (Synthes Spine, Inc.).

The Dynesys system is composed of titanium alloy pedicle screws, polyester cords, and polycarbonate-urethane (PCU) spacers. It is fixed in place using standard pedicle screws made of a titanium alloy, and the entire system is stabilized by polyester cords that connect the screw heads through a hollow spacer and hold the screws in place.

The NFlex system consists of polyaxial titanium alloy pedicle screws that are fixed to a semi-rigid polycarbonate urethane-sleeved rod. The integrated PCU spacer is surrounded by a central titanium ring, to which a pedicle screw is locked. The rod may be attached to pedicle screws in the standard fashion. The PCU spacer is not bonded to the titanium components of the rod. Therefore, when the pedicle-to-pedicle distances increases and decreases during the flexion and extension, the rod is able to elongate and compress to accommodate this change. It can also toggle to accommodate angulation.

#### Surgical procedure

Reoperations were performed using previous midline incisions in a neutral prone position. After exposure of the previous fusion segment and index segment, manual distraction was undertaken to determine pseudarthrosis of the fusion segment. If gross motion was not observed, existing rods and screws from the fusion segment were removed, after which adjacent segments which were symptomatic were decompressed via hemilaminectomy or total laminectomy. Additional foraminal decompression also was performed in cases of foraminal stenosis. But additional discectomy was not performed even in combined with degenerative disc disease (DDD). During decompression, the integrity of the facet joint was carefully maintained for the non-fusion dynamic stabilization system. After adequate decompression, dynamic stabilization with Dynesys or NFlex was applied.

#### Clinical evaluation

Through patients’ medical records, pain was mea-
sured by the Visual Analog Scale (VAS, 0-10) and functional outcome was assessed by the Korean version of Oswestry Disability Index (ODI, 0-100%) excluding the item on sexual activity. Clinical parameters were measured before the non-fusion dynamic stabilization surgery and during every postoperative year. Clinical ASP was defined as a condition in which a patient was relieved of symptoms for at least six months after the non-fusion surgery, with newly developed symptoms later, which were compatible with the lesions in adjacent segments demonstrated in radiological images. In addition, medication via pain analgesics such as non-steroid anti-inflammatory drugs (NSAIDs) or Gabapentin was evaluated before the operation and upon the last follow-up.

**Radiologic evaluation**

Disc degeneration, central canal stenosis, and facet joint degeneration were graded on magnetic resonance images (MRI) before the initial fusion surgery, before the non-fusion dynamic stabilization surgery and in the last evaluated images. They were evaluated using the Pfirrmann disc degeneration grade system (grade, I-V), a new MRI grade for central canal stenosis (grade, 0-3) and in terms of the Weishaupt facet joint degeneration grade (grade, 0-3, Table 1). In addition, instances of disc degeneration from L1-2 to L5-S1 were sequentially evaluated on lateral radiographs (Table 2). The range of motion (ROM) calculated the difference between the flexion-extension dynamic radiographs at T12-S1 segments before and after non-fusion dynamic stabilization surgery. Radiologic ASP included disc space narrowing (>2mm loss of the posterior disc height), spur formation, spondylolisthesis (anterior or posterior slip of the vertebra by > 2mm), and vacuum phenomenon, comparing each radiographs. Radiologic abnormalities such as a radiolucent line around the pedicle screw of the dynamic stabilization system and instrument failure such as a fracture of a screw or a rod were evaluated at each follow-up on plain radiographs. Radiologic data was investigated using a picture archiving and communication system (PACS, M-view, version 5.483, Infinitt Healthcare, Seoul, Korea).

**Statistical analysis**

The Mann-Whitney test and Wilcoxon signed rank tests were used for continuous variables, and Fish-er’s exact test was used for categorical variables. Sta-
Statistical significance was defined as a p-value of less than 0.05. Analyses were performed using SPSS version 19.0 software (IBM, Armonk, New York, USA).

Results

Fifteen patients who had previously undergone lumbar fusion surgery and then later underwent non-fusion dynamic stabilization surgery participated in this study. The gender distribution was thirteen females and two males, the mean age was 56.2 ± 6.9 years (range, 43-67) upon the prior fusion surgery, 62.1 ± 6.4 years (range, 46-70) upon the non-fusion surgery, and 68.5 ± 6.8 years (range, 52-77) at the final follow-up (Table 3). The previous fusion segment was mostly the L4-5-S1 (6, 40.0%) followed by L4-5 segment (5, 33.3%), L3-4 (2, 12.5%), L3-4-5 (1, 6.6%) and L2-3 (1, 6.6%). Primary pathology was lumbar spinal stenosis in all cases and it associated with DDD in six patients (40.0%) and degenerative spondylolisthesis in three patients (20.0%). The fusion technique was posterior lumbar interbody fusion (PLIF) with pedicle screw fixation (PSF) in nine patients (60.0%), PLIF without PSF in 4 (26.6%), PSF with posterolateral fusion (PLF) in 1 (6.6%), and anterior lumbar interbody fusion (ALIF) without PSF in 1 (6.6%). The mean pain-free period after fusion was 43.4 ± 30.3 months (range, 0-96), and the mean symptomatic period before non-fusion surgery was 38.1 ± 23.7 months (range, 2-84). Five patients complained about persistent symptoms after fusion surgery, and all patients had continued with their medical treatment, using on average 2.2 types of analgesics. Consequently, the time interval between the prior fusion surgery and the later non-fusion surgery was 67.0 ± 36.9 months (range, 6-120). As ten of the 15 patients showed ASP at L3-4, it was indicated as the most common proximal adjacent segment from previous fusion. The pathology of ASP was spinal stenosis in all cases, and it was associated with DDD (8, 53.3%) and spondylolisthesis (2, 13.3%). Non-fusion dynamic stabilization surgery was applied at L3-4 (7, 46.6%), L4-5 (3, 20.0%), L1-2-3 (1, 6.6%), L2-3-4 (1, 6.6%), L3-4-5 (1, 6.6%), L2-3-4-5 (1, 6.6%) and L2-3 (1, 6.6%). The Dynesys system (Figure 1) was used on eleven patients while the NFlex system (Figure 2) was used on four patients. Mean T-score at L1-4 on the DEXA bone densitometry was -0.5 ± 1.3 (range, -2.1 – 2.1).
<table>
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<th>No.</th>
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<th>Age at non-fusion surgery (yrs)</th>
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<th>Pain free period after fusion (m)</th>
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DDD: degenerative disc disease; SPL: spondylolisthesis; +: developed; -: absent.
Clinical outcomes
Mean follow-up was conducted for 44.8 ± 25.0 months (range, 12-79). Before the non-fusion surgery, the VAS was checked and was found to be 7.4 ± 1.8 (range, 5-10); it had a tendency to decrease after non-fusion surgery, and it showed a significantly decrease to 4.2 ± 1.1 (range, 3-5) upon the last evaluation (p= 0.027, Figure 3A). ODI was preoperatively 58.5 ± 16.9% (range, 35.5-88.8), and it changed to 41.3 ± 22.8% (range, 11.1-60.0, p=0.018, Figure 3B). However, three patients complained about persistent pain after their non-fusion surgery, and four patients (26.6%) had a relapse of pain in the back and leg after a mean of 30 months after non-fusion surgery (30.0 ± 11.3 months, range, 20-43). Upon the last follow-up, four patients had not taken analgesics, and the remaining eleven patients could reduce the number of their medications to a mean 1.2 analgesics. This was a statistically significant decrease compared to the amount before the non-fusion surgery (p=0.004). Persistent pain after the fusion and non-fusion surgeries, as well as relapsed pain after the non-fusion surgery, did not affect the differences in both the VAS and ODI values (all p > 0.05). No patient underwent additional surgery for ASP after undergoing non-fusion dynamic stabilization surgery.

MRI changes according to the surgical procedure
The last MRI was obtained at 36.2 ± 23.8 months (range, 12-72) after non-fusion surgery. The Pfirrmann disc degeneration grades were respectively 3.0, 3.5, 3.2, 3.5 and 3.2 at segments L1-2 to L5-S1 before fusion surgery; these were 3.3, 3.6, 4.1, 4.0 and 3.8 at each respective segment before non-fusion surgery (Figure 4A and Figure 5). Only the intervertebral disc at L3-4 between the fusion and non-fusion surgeries had degenerated to a statistically significant degree (p=0.046). Upon the last MRI, the disc degeneration grades were 4.0, 4.2, 4.2, 4.2 and 4.0 at each respective segment, and the change at L1-2 between non-fusion surgery and the last MRI showed statistical significance (p=0.032), whereas other segments had not changed (all p > 0.05). Through central canal stenosis was respectively graded as 0, 0.3, 1.0, 1.1 and 0.2 at segments L1-2 to L5-S1 before fu-
sion surgery, these values were respectively 0.1, 0.7, 1.5, 0.5 and 0.2 before non-fusion surgery (Figure 4B), and central stenosis at L3-4 had significantly worsened (p=0.041). Stenosis at L4-5 was decompressed from the fusion surgery, but this did not show statistical significance (p=0.114). Stenosis at L3-4 was sufficiently decompressed after non-fusion surgery (p=0.041), and the changes in other segmental stenosis conditions upon the last MRI were not significant. Facet joints showed gradual degeneration at points before the fusion to after the non-fusion surgeries, but these changes were not significant at all segments (all P > 0.05, Figure 4C).

Radiologic changes on lateral radiographs
The disc degeneration changes according to lateral radiographs are shown in Figure 6. Before fusion surgery, disc degeneration was observed as follows: 0.9, 1.9, 2.0, 2.4 and 1.6 from L1-2 to L5-S1. Before non-fusion surgery, these values were respectively 1.2, 1.6, 2.8, 2.2 and 1.7, and the degree of disc degeneration at L3-4 showed a significant change (p=0.046). As each disc gradually degenerated, disc degeneration was graded at 3.0, 3.3, 2.6, 3.0 and 1.6 at 36 months after non-fusion surgery. The most frequently involved segment, L3-4, was not changed after non-fusion surgery. Disc degeneration was observed at L1-2 and L2-3, but neither was not statistically significant (p=0.083 and p=0.083, respectively). ROM at T12-S1 was 22.3˚ ± 22.3 before non-fusion surgery and it was measured 19.1˚ ± 20.3, 20.4˚ ± 17.8 and 16.0˚ ± 7.2 at each annular follow-up period (p=0.180). Based on the definition of radiologic ASP, it was observed in five patients (33.3%) at 30.0 ± 9.5 months (range, 24-40) after the non-fusion surgery. These radiologic features showed disc space narrowing at L1-2 and L2-3 in all cases as a proximal segment. In terms of radiologic abnormalities, screw or NFlex rod fractures were not observed, but a radiolucent line around a screw was identified in five patients (33.3%) with seven screws at the last radiographs.

Discussion
Surgical outcomes of ASP treated with the non-fusion dynamic stabilization system
Many spine surgeons have an interest in managing ASP in patients who undergo fusion surgery and in knowing which surgical method is most effective to prevent ASP. First, additional fusion extension surgery for ASP reportedly shows good clinical outcomes. Five fusion extension studies demonstrated this in patients with a mean age ranging from 52 to 61
years, with males making up 23% to 50% of the study populations. The time interval between fusion extension for ASP and the previous fusion surgery ranged from 4.8 to 11.5 years. Although the clinical outcome measurement methods among these five fusion studies varied, satisfactory results were noted in 76.9% - 85% of cases, and postoperative pain relief was identified in 78.6%. In a recent study about revision lumbar surgery in elderly patients (age > 65 years) with symptomatic pseudarthrosis, ASP, or same-level recurrent stenosis, VAS values for back pain and two-year ODI showed significant improvements two years after surgery. However, informal outcome measures taking into account pain, medication use, and return to work showed a good outcome in 35.7% of cases. Two years postoperatively, only 38% and 40% of patients reached the minimum clinically important difference (MCID) threshold for the ODI and the 36-item short-form health survey (SF-36) physical component scale, respectively. Moreover, improvement from the previous surgery was the most predictive factor for subsequent improvement after fusion extension for ASP. Additionally, long revision fusion surgery has overall 34.4% of major complications and associated risk factors were age > 60 years, medical comorbidities and obesity. In two studies of ASP treated with decompression only, patients showed good to excellent results in 57.7% and 64.2% of cases. However, decompression led to increased reoperation and revision rates, and in a meta-analysis, laminectomy alone for the treatment of stenosis with degenerative spondylolisthesis was associated with a 31% incidence of slip progression. Instability and recurrence of symptomatic stenosis are both reported complications of laminectomy alone for the treatment of degenerative lumbar scoliosis and stenosis. One study reported results of patients with ASP treated with a motion-preserving technique using total disc arthroplasty (TDR). Twenty patients in whom symptomatic ASP after lumbar fusion underwent ProDisc lumbar TDR, after which significant improvements in patient satisfaction and disability scores were observed three months postoperatively; these scores were maintained at a two-year follow-up.

Through there is no report on ASP treated with a motion-preserving technique using a pedicle-based dynamic stabilization system, the present study is the first to report the surgical outcome of ASP treated with non-dynamic stabilization system surgery with mid-term follow-ups. As surgical indications for non-fusion dynamic stabilization surgery were extended, the corresponding surgeon attempted to apply this non-fusion dynamic stabilization system to patients who suffered from ASP after fusion surgery. ASP after fusion surgery was identified with disc degeneration and hypertrophy of the ligamentum flavum, causing adjacent segment stenosis, without any significant deformity or instability. Hence, surgery was planned to remove previous inserted rods and screws, to perform sufficient decompression with preservation of integrity of intervertebral disc and facet joints, and to apply the non-fusion dynamic stabilization system to the symptomatic segment. Radiographical important difference (MCID) threshold for the ODI and the 36-item short-form health survey (SF-36) physical component scale, respectively. Moreover, improvement from the previous surgery was the most predictive factor for subsequent improvement after fusion extension for ASP. Additionally, long revision fusion surgery has overall 34.4% of major complications and associated risk factors were age > 60 years, medical comorbidities and obesity. In two studies of ASP treated with decompression only, patients showed good to excellent results in 57.7% and 64.2% of cases. However, decompression led to increased reoperation and revision rates, and in a meta-analysis, laminectomy alone for the treatment of stenosis with degenerative spondylolisthesis was associated with a 31% incidence of slip progression. Instability and recurrence of symptomatic stenosis are both reported complications of laminectomy alone for the treatment of degenerative lumbar scoliosis and stenosis. One study reported results of patients with ASP treated with a motion-preserving technique using total disc arthroplasty (TDR). Twenty patients in whom symptomatic ASP after lumbar fusion underwent ProDisc lumbar TDR, after which significant improvements in patient satisfaction and disability scores were observed three months postoperatively; these scores were maintained at a two-year follow-up.

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ologic changes through MRI and lateral radiographs revealed the progression of disc degeneration and central stenosis at adjacent segments, especially at L3-4 as a proximal segment upon the development of ASP after the fusion procedure. The overall clinical outcome in terms of VAS and ODI showed great improvements after non-fusion surgery, and reductions in the use of pain medication were observed upon the last follow-up. However, five patients complained of persistent pain after the previous fusion surgery, and among them, four patients presented with persistent pain or relapsed pain even after non-fusion surgery. One additional patient presented with persistent pain after non-fusion surgery and two patients experienced pain relapses within 30 months after non-fusion surgery. Although much improvement in terms of pain scores and reductions in pain medication was observed, functional outcomes measured by ODI showed moderate disabilities which were not comparable with our previous clinical experiences with the dynamic stabilization system. In research about poor ODI outcomes among 1054 patients who underwent lumbar spinal fusion, younger men, obese patients, smokers, those with multiple medical comorbidities, those receiving workers compensation, those with psychosocial stressors, and those who had undergone revision lumbar surgery were associated with poor functional outcomes. And revision surgery for adult deformity surgery also reported significantly lower clinical outcomes in terms of function and pain. Similarly, the authors felt that unsatisfactory functional outcomes may be associated with revision surgery.

**ASP development after the use of the non-fusion dynamic stabilization system**

Through the objective of non-fusion dynamic stabilization is to maintain the normal physiologic range of motion (ROM) and reduce disc and facet joint stress, these theoretically biomechanical properties suggest that this procedure should produce better clinical outcomes, especially regarding ASP prevention. Successful clinical outcomes in patients who undergo non-fusion dynamic stabilization have been reported, but the long-term effect on ASP after the application of a dynamic stabilization system has not been thoroughly addressed in a clinical series. In the present study, changes in commodities disc, spinal canal and facet joint were in the form of gradual degeneration after non-fusion dynamic surgery at all lumbar segments, and commodities operative segment treated with the non-fusion dynamic stabilization system was preserved without statistical significance. Nonetheless, significant changes were found in the intervertebral disc at L1-2 according to postoperative MRI follow-ups. On lateral radiographs, disc degeneration grades were increased after non-fusion surgery, but these changes were also not statistically significant when comparing grades before non-fusion surgery and 36 months postoperatively. However, radiologic ASP was observed in five patients (33.3%) within 30 months after non-fusion dynamic stabilization surgery. The main characteristic of ASP was disc space narrowing at the segment proximal to the non-fusion instrumented segment; these segments were L1-2 in one patient and L2-3 in four patients. Although the changes at the L1-2 and L2-3 segments were not statistically significant, degeneration in the disc and in the facet joint leading to central stenosis at the corresponding segments was identified by means of MRI and plain radiographs.

Much debate regarding ASP has centered on how much of the degeneration is actually related to the fused segment and how much is related to the natural history of the aging spine. According to a recent systemic review about the natural history of the degeneration of the spine, biomechanical effects after fusion may accelerate pathologic changes at adjacent segments, and radiologic ASP may occur at a higher rate relative to the natural history. We observed the sequential development of ASP after fusion surgery followed by ASP after non-fusion surgery. Therefore, the authors agree that there is likely an adverse effect of instrumented surgery on the spine on the development of ASP. Even after non-fusion dynamic stabilization, ASP developed at a rate of 33.3% within 30 months after non-fusion surgery. The mean age at surgery differed by six years between the prior fusion and the later non-fusion surgeries, but ASP developed in a similar period after both surgeries. Considering degeneration caused by the aging process, the author suggests that the non-fusion dynamic stabilization system cannot prevent the development of ASP but that the current system may delay this. Several biomechanical studies of current dynamic stabili-
lization systems have revealed non-physiologic effects, such as insufficient ROM and cases of an abnormal instantaneous center of rotation (COR) compared to an intact spine.\textsuperscript{34-36} Other authors have reported that the stiffness resulting from the use of the Dynesys system is identical to that resulting from rigid fixation. All of these studies suggest that the Dynesys system is limited when used to simulate the normal spinal motion of an intact spine. The biomechanical discrepancies and design characteristics of the dynamic stabilization system may have a flaw with regard to the prevention of ASP. In addition, several reports reported that screw loosening was not significant problem during dynamic stabilization but that the risk factors and long-term effects should be investigated.

Study limitation
This study reported single surgeon’s clinical experiences with a non-fusion dynamic stabilization system for ASP involving a small number of patients. As the mean follow-up period was 44.8 months, this relatively short follow-up time is also a limitation when formulating any conclusion. A comparative study of decompression alone, a non-fusion dynamic stabilization system and fusion extension surgery should be conducted for useful results in patients after they have undergone lumbar fusion, but with a relative small number of patients, we could not plan such a study. While two types of dynamic stabilization systems were used in the present study, four patients in Dynesys system group and one patient in the NFlex system group showed radiologic ASP. A better physiologic dynamic device could not be determined given our small study population, and a prospective randomized study comparing different types of dynamic stabilization systems should be designed. Hence, this study is more of a preliminary report of a series of cases regarding the application of a non-fusion dynamic stabilization system for ASP after lumbar fusion. Although ASP after non-fusion dynamic stabilization surgery was still observed, these devices may be able to delay the development of ASP. Furthermore, additional physiologic devices for motion preservation should be devised and tested.

Conclusion
The authors identified that non-fusion dynamic stabilization surgery was a safe procedure corresponding to fusion surgery, even in elderly patients with ASP. ASP after non-fusion dynamic stabilization surgery was still observed, but the clinical outcomes observed significant improvements, and these devices may be able to delay the development of ASP. Radiologic ASP was observed in five patients within 30 months after non-fusion surgery, but no additional surgery for ASP was conducted in all cases. Long-term studies are necessary to determine the effectiveness of these systems on the prevention of ASP after non-fusion dynamic stabilization surgery.

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Disclosures
The authors report no conflict of interest concerning the materials or methods used in this study or the findings described in this paper. No benefits in any form have been or will be received from any commercial party related directly or indirectly to the subject of this manuscript.

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