CASE SERIES

Contemporary utilization of three‑column osteotomy techniques in a prospective complex spinal deformity multicenter database: implications on full‑body alignment and perioperative course

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Received: 23 January 2024 / Accepted: 23 May 2024 © The Author(s), under exclusive licence to Scoliosis Research Society 2024

Abstract

Background Research has focused on the increased correction from a three-column osteotomy (3CO) during adult spinal deformity (ASD) surgery. However, an in-depth analysis on the performance of a 3CO in a cohort of complex spinal deformity cases has not been described.

Study design/setting This is a retrospective study on a prospectively enrolled, complex ASD database.

Purpose This study aimed to determine if three-column osteotomies demonstrate superior beneft in correction of complex sagittal deformity at the cost of increased perioperative complications.

Methods Surgical complex adult spinal deformity patients were included and grouped into thoracolumbar 3COs compared to those who did not have a 3CO (No 3CO) (remaining cohort). Rigid deformity was defned as ΔLL less than 33% from standing to supine. Severe deformity was defined as global (SVA>70 mm) or C7-PL>70 mm, or lumbopelvic (PI-LL>30°). Means comparison tests assessed correction by 3CO grade/location. Multivariate analysis controlling for baseline deformity evaluated outcomes up to six weeks compared to No 3CO.

Results 648 patients were included (Mean age 61 ± 14.6 years, BMI 27.55 ± 5.8 kg/m², levels fused: 12.6 ± 3.8). 126 underwent 3CO, a 20% higher usage than historical cohorts. 3COs were older, frail, and more likely to undergo revision (OR 5.2, 95% CI [2.6–10.6]; p<.001). 3COs were more likely to present with both severe global/lumbopelvic deformity (OR 4), 62.4% being rigid. 3COs had greater use of secondary rods (OR 4st) and incurred 4 times greater risk for: massive blood loss (>3500 mL), longer LOS, SICU admission, perioperative wound and spine-related complications, and neurologic complications when performed below L3. 3COs had similar HRQL beneft, but higher perioperative opioid use. Mean segmental correction increased by grade (G3–21; G4–24; G5–27) and was $4 \times$ greater than low-grade osteotomies, especially below L3 (OR 12). 3COs achieved $2 \times$ greater spinopelvic correction. Higher grades properly distributed lordosis 50% of the time except L5. Pelvic compensation and non-response were relieved more often with increasing grade, with greater correction in all lower extremity parameters $(p < 0.01)$. Due to the increased rate of complications, 3COs trended toward higher perioperative cost (\$42,806 vs. \$40,046, $p = .086$).

Conclusion Three-column osteotomy usage in contemporary complex spinal deformities is generally limited to more disabled individuals undergoing the most severe sagittal and coronal realignment procedures. While there is an increased perioperative cost and prolongation of length of stay with usage, these techniques represent the most powerful realignment techniques available with a dramatic impact on normalization at operative levels and reciprocal changes.

Keywords Adult spine deformity · Sagittal alignment · Osteotomy · Three-column osteotomy

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Introduction

Spine surgery has seen a wave of infuential surgical strategies, minimally invasive techniques, and comprehensive, individualized preoperative and postoperative care, thus expanding the population eligible for surgical intervention [\[1–](#page-7-0)[6\]](#page-7-1). As management is optimized for patients with higher comorbidities and frailty, so too is the correction for even more complex deformities $[7–11]$ $[7–11]$ $[7–11]$. Multi-dimensional and severe deformity has seen dramatic improvements in corrective strategies, along with tailored goals to ft a patient's realignment needs to be based both on fxed, individualized parameters and relative to their age [[12](#page-7-4)–[15](#page-7-5)]. Historically, patients presenting with complex adult spinal deformities are inherently at greater risk for postoperative complications, diminished improvement, and future reoperations due to mechanical or radiographic complications. Therefore, it is important to assess the current techniques employed within this cohort to improve the outcomes for such invasive and taxing procedures.

Numerous prior studies have established the correlation between reaching alignment goals and patient outcomes [\[7\]](#page-7-2). Additionally, failure to meet alignment goals has been shown to potentially correlate with complications such as rod fracture and PJK [\[14,](#page-7-6) [15\]](#page-7-5). Complex ASD patients present an additional challenge in that they require signifcant correction relative to standard ASD patients; therefore, the use of posterior column osteotomies, multilevel interbody fusions, or contoured rods are not sufficient for $30 + \text{degree}$ corrections.

Three-column osteotomies (3COs), consisting of pedicle subtraction osteotomies (PSOs) or vertebral column resections (VCRs), allow for a greater degree of correction than other less invasive techniques and may be necessary in the case of severe, rigid deformity and fat-back syndrome. However, complication rates for 3COs were previously found to be as high as 60% with increasing rates seen in older patients (> 60 years old), those with > 1 3CO, a thoracic 3CO (vs. lumbar or sacral), or massive blood loss $(>4 L) (3-6).$

Although the rates of 3CO usage are declining overall, this technique is still a necessary option for patients with severe, rigid deformities [[16\]](#page-7-7). Previous literature has described the greater correction seen with 3CO in typical adult spinal deformity populations [\[17\]](#page-7-8). We sought to analyze the perioperative outcomes of 3COs particularly in patients with highly complex deformities. We hypothesized that these patients would experience more perioperative complications but greater correction of alignment and reciprocal changes.

Materials and methods

Study design and inclusion criteria

This study was a retrospective review of the prospectively enrolled, multicenter complex adult spinal deformity database from 2018 to 2022. This dataset collects clinical, surgical, and outcome data from 18 participating centers across the United States and Canada. Patients aged 18 or older included for retrospective review enrolled in the registry from 2020 to present with either radiographic evidence, procedural or geriatric criteria characteristic of complex adult spinal deformity (ASD). Radiographic evidence for complex ASD is defined as follows: $PI-LL \geq 25^{\circ}$, T1PA $\geq 30^{\circ}$, SVA > 15 cm, thoracic scoliosis $\geq 70^{\circ}$, thoracolumbar/lumbar scoliosis $\geq 50^{\circ}$, or global coronal malalignment > 7 cm. Procedural details characteristic of complex ASD include 3-column osteotomies (3COs) and/or anterior column reconstruction (ACR) of the spine or posterior spinal fusion > 12 levels. Geriatric complex ASD criteria are defned as age>65 years and a minimum of seven levels of spinal instrumentation during surgery with an intention to treat deformity. We included operative complex adult spinal deformity patients who had complete radiographic and health-related quality of life (HRQL) data at baseline and perioperative (up to six weeks) followup in the current maturity of this dataset.

Data collection

Demographic data that were abstracted for eligible individuals consisted of age, biological sex, body mass index (BMI), history of prior fusion, *Passias* et al. modifed adult spinal deformity frailty index (modifed ASD-FI), and baseline comorbidities categorized using the Charlson Comorbidity Index (CCI) [\[4,](#page-7-9) [5](#page-7-10)]. Surgical parameters consisted of levels fused, operative time, length of stay, surgical approach, use of decompressions, and osteotomies. A standardized complications reporting form was completed for the perioperative time interval for each clinical follow-up and at any point the site became aware of a new complication or adverse event. The de-identifed data from each center were sent to a central site where the collective datasets were summarized and analyzed and the complications were reviewed. Patient-reported outcome measures, prospectively collected at baseline and followup intervals, included modifed Oswestry Disability Index for low back pain (ODI), Scoliosis Research Society Questionnaire 22r (SRS-22r), Veterans RAND-12 (VR-12), numeric pain rating scale (NRS), and the Patient-Reported Outcomes Measurement Information System (PROMIS)

Domains––PROMIS anxiety, depression, pain interference, physical function, and social satisfaction. Outcome assessments were completed via patient surveys at baseline and during subsequent follow-up encounters up to six weeks following surgery. Abbreviations for each variable are displayed in Table [1](#page-2-0).

Radiographic data collection

Full-length free-standing lateral spine radiographs (36 inch cassette) were collected and assessed at baseline and follow-up. Radiographs will be reviewed for postoperative correction and revision surgery. All radiographic analyses of the spinal axis will be performed using full spine EOS

Table 1 Table of abbreviations

Abbreviation	Definition		
3CO	Three-column osteotomy		
ACR	Anterior column reconstruction		
AА	Ankle angle		
ASD	Adult spinal deformity		
ASD-FI	Adult spinal deformity frailty index		
BMI	Body mass index		
CCI	Charlson comorbidity index		
EBL	Estimated blood loss		
$%$ EBV	Percent of estimated blood volume		
GSA	Global spinal angle		
HAC	Hospital-acquired condition		
HRQL	Health-related quality of life		
KA	Knee angle		
\mathbf{I}	Lumbar lordosis		
LOS	Length of stay		
MCID	Minimal clinically importance difference		
NRS	Numeric pain rating scale		
ODI	Oswestry disability index		
РI	Pelvic incidence		
$PI-L$	Mismatch between pelvic incidence and lumbar lordosis		
PROMIS	Patient-reported outcomes measurement information system		
PS	Pelvic shift		
PSO	Pedicle subtraction osteotomy		
PT	Pelvic tilt		
SFA	Sacrofemoral angle		
SICU	Surgical intensive care unit		
$SRS-22r$	Scoliosis research society questionnaire 22r		
SVA	Sagittal vertical axis		
TК	Thoracic kyphosis		
T ₁ PA	T1 pelvic angle		
VCR	Vertebral column resection		
VR-12	Veterans RAND-12		

imaging that includes sagittal and coronal visualization of the top of the skull to the bottom of the foot. Sagittal and coronal radiographic parameters will obtained from the EOS radiographs using appropriate radiographic imaging and measurement software including Spineview®, (Laboratory of Biomechanics, Paris, France), and Surgimap® as previously published. [\[1](#page-7-0)[–3](#page-7-11)]. Spinopelvic radiographic parameters measured were pelvic tilt (PT), pelvic incidence (PI), sagittal vertical axis (SVA), thoracic kyphosis (TK, T4-12), T1 pelvic angle (T1PA), lumbar lordosis (LL, T12-S1), and mismatch between pelvic incidence and lumbar lordosis (PI-LL). Lumbar fexibility was assessed by supine radiographs. Lower extremity parameters measured were sacrofemoral angle (SFA), knee angle, ankle angle (AA), pelvic shift (PS), and Global Spinal Angle (GSA).

Clinical outcomes

To evaluate improvement in outcomes, minimal clinically importance difference (MCID) thresholds were utilized based on published values in the literature: ODI (12.8), SRS-Pain (0.587), SRS-Mental (0.42), SRS-Activity (0.375), and SRS-Appearance (0.8) [[6](#page-7-1)[–11](#page-7-3)].

Radiographic assessment

Changes in L1-S1 lordosis<33% between their preoperative standing and supine radiographs were considered "rigid." Severe deformity was classified as global $(SVA > 80$ mm), lumbopelvic (PI-LL $> 30^{\circ}$), and coronal (C7 plumb $line > 70$ mm).

Complication assessment

The reported complications were classifed as minor or major, with complications that involved invasive intervention or prolonged or permanent morbidity or mortality classifed as major. Complications were grouped based on time of occurrence as perioperative (within 90 days of surgery, including hospital-acquired conditions [HACs; DVT/PE, UTI, deep/superficial infection]) and longer term (recorded from 90 days to at least two years following surgery) [[13](#page-7-12)]. Medical complications were defned as cardiovascular, pulmonary, musculoskeletal, central nervous system, gastrointestinal, wound, or neurological complications not directly related to the procedure. Spine complications were defned as any complication related to the spine, implant, or radiographic alignment.

Group categorization

Patients receiving a 3CO (grade 3 or above Schwab classifcation osteotomy: pedicle subtraction osteotomy [PSO], extended pedicle subtraction osteotomy, vertebral column resection [VCR], multiple-level VCR) performed between T10 and L5 were compared with No 3CO patients (remaining cohort). Patients undergoing a 3CO were stratifed based on Schwab osteotomy grade (3–6) and location of the three-column osteotomy. The thoracolumbar three-column osteotomies within this cohort were performed by 13 Scoliosis research society active fellows with $10-30$ + years of experience in spinal deformity surgery.

Cost calculation

The PearlDiver database was utilized to calculate costs using job order cost accounting ("charge analysis"). Refecting both Medicare reimbursement and private insurance, the Pearl-Diver database is one of the most comprehensive datasets with access to Medicare reimbursement charges, outcome data, and trends. Using mean costs associated with procedures based on 2018 adult spinal deformity diagnosis-related groups, procedural costs for cases, cases with complications and comorbidities (CC), major complications and comorbidities (MCC), and revisions were determined according to CMS.gov manual defnitions [[18\]](#page-7-13). Our estimates for two-year reimbursement consisted of a standardized determination using regression analysis of Medicare pay scales for all services rendered within a 30-day window, including costs of postoperative complications, outpatient healthcare encounters, revisions, and medical-related readmissions, as per previously published methods [\[18](#page-7-13)[–23\]](#page-7-14).

Statistical analysis

The primary outcomes included surgical details, hospital stay outcomes, complications, HACs, radiographic global and segmental correction, reoperations, and clinical HRQL outcomes. Baseline demographic, radiographic, and clinical data were compared between the cohorts using chi-squared and t-tests for categorical and continuous variables, respectively. Means comparison tests assessed spinopelvic correction by location and grade of 3CO. Multivariate analysis of patients controlling for baseline PI-LL and the number of levels fused evaluated complication rates, radiographic, and patient-reported outcomes. Statistical signifcance for all analyses corresponded to a p-value less than 0.05. All statistical analyses were conducted using SPSS, version 28.1 (Armonk, NY).

Results

Patient demographics

Of 381 total patients, there were 249 complex ASD patients eligible and meeting inclusion criteria with full baseline and perioperative data. Mean cohort demographics were as follows: age of 61.0 ± 14.6 years, BMI of 27.5 ± 5.8 kg/m², CCI of 1.0 ± 1.5 , and modified ASD frailty index of 7.4 ± 4.4 .

Surgical details

During surgery, patients endured a mean operative time of 469 ± 160 min and estimated blood loss (EBL) of 1629 ± 1287 mL or percent of estimated blood volume (%EBV) of 32.6 ± 25.7 %, while 59% underwent a decompression and 72% underwent an osteotomy. Three-column osteotomies (3CO) were performed in 20.5% of procedures, with a mean 12.6 ± 3.8 levels fused. Regarding surgical approach, 83% were posterior-only and 17% were combined.

Cohort radiographic assessment

Patients had the following mean baseline radiographic measurements: SVA: 67.9 ± 79.8 mm, PI-LL: $16.3 \pm 23.5^{\circ}$, PI: 54.6 \pm 13.0°, T1PA: 24.2 \pm 14.2°, PT: 24.5 \pm 12.0°, GSA: 5.3 \pm 6.1°, KA: 5.5 \pm 9.3°, and AA: 5.2 \pm 4.5°. The mean baseline GAP score of the cohort was 8.3 ± 4.2 . Upon correction, the cohort showed improvement in all parameters (all $p < 0.001$). Patients had the following mean six-week radiographic measurements: $SVA: 23.8 \pm 41.7$ mm, PI-LL: $3.4 \pm 13.7^{\circ}$, T1PA: $16.8 \pm 10.0^{\circ}$, PT: $21.1 \pm 10.6^{\circ}$, global sagittal angle (GSA): $1.8 \pm 3.8^\circ$, Knee Angle (KA): $2.9 \pm 7.5^\circ$, and Ankle Angle (AA): $4.9 \pm 4.4^{\circ}$. The mean six-week GAP score was 5.3 ± 3.6 , with 22.6% being proportioned.

Categorization

Of those included, 51 patients (21%) had a 3CO. This is a 20% greater usage than earlier ASD databases (17%).

Baseline demographic diferences

The demographic differences between groups are displayed in Table [2.](#page-4-0) Patients undergoing a 3CO were older $(65.6 \text{ vs. } 60.9, p=0.023)$, with frailty increasing with 3CO grade $(r=0.537, p<0.001)$. Compared to No 3COs, 3COs were more likely to present as a revision (OR 5.2, 95% CI $[2.6-10.6]$; p < 0.001).

Baseline radiographic diferences

The baseline radiographic differences between groups are displayed in Table [2](#page-4-0). Patients undergoing a 3CO were more likely to present with severe deformity in PI-LL (OR 10.5, 95% CI [4.5–24.6]; p<0.001) and PT (OR 5.1, 95% CI $[2.7-9.8]$; $p < 0.001$). Patients undergoing a 3CO more often presented with the most complex deformities, more likely to present with both severe global and lumbopelvic **Table 2** Baseline and surgical comparison between 3CO and non-3CO groups

BMI body mass index, *CCI* Charlson comorbidity index, *TKA* total knee arthroplasty, *THA* total hip arthroplasty, *PJK* proximal junctional kyphosis, *SICU* surgical intensive care unit, *SNF* skilled nursing facility, *PI* pelvic incidence, *PT* pelvic tilt, *LL* lumbar lordosis, *SVA* sagittal vertical axis, *T1PA* T1 pelvic angle, *L1PA* L1 pelvic angle, *L4PA* L4 pelvic angle, *LDI* lordosis distribution index, *SFA* sacrofemoral angle, *KA* knee

Table 2 (continued)

angle, *AA* ankle angle, *GSA* global spinal angle, *ODI* Oswestry disability index, *PCS* physical component score, *MCS* mental component score

	3CO(%)	No $3CO(%)$	p-value
90-day complications			
Any	58.8	52.2	.423
Intraoperative	27.5	12.6	.009
Medical	21.6	25.8	.539
Cardiopulmonary	13.7	11.6	.681
Central nervous system	0.0	3.6	.175
Gastrointestinal	5.9	6.6	.860
Genitourinary	3.9	3.0	.749
Wound	19.6	5.6	.001
Neurological	27.5	18.7	.168
Spine-related	54.9	37.9	.028
PJK.	29.4	24.7	.499
PJF	3.9	2.6	.610
Reoperation	25.5	15.2	.082
Mortality	0.0	1.0	.613

PJK proximal junctional kyphosis, *PJF* proximal junctional failure

deformity compared to No 3CO (OR 11.9, 95% CI $[5.9-24.2]$; p < 0.001).

In‑hospital, complication, and clinical improvement comparison

The surgical details between groups are displayed in Table [2.](#page-4-0) The total cohort length of stay is 7.0 ± 6.2 days. 3COs had four times greater use of a secondary rod, incurring a four times greater risk of massive blood loss (>3500 mL), along with longer length of stay, and higher rates of SICU admission, perioperative wound, and spine-related complications, and, specifically, neuro complications when performed below L3 (32.4% vs. 18.7%) (Table [3\)](#page-5-0). 3COs saw similar benefts in HRQLs, although less resolution of baseline sensory and motor deficits and higher opioid usage perioperatively (Table [4](#page-5-1)). Of note, when comparing only patients undergoing primary $3COs$ (n=12) to those not undergoing a 3CO, there were no diferences in any complication rates or clinical improvement measures (all $p > 0.1$).

Six‑week radiographic assessment

Upon correction, degree of segmental correction increased by 3 degrees with increased grade of 3CO (G3–20.5; G4–23.4; G5–26.6) and demonstrated at least 4 times greater correction at each level compared to lower-grade osteotomies, including 12 times greater correction at L4 and L5. 3COs achieved double the correction in lumbopelvic and

ODI Oswestry disability index, *MCID* minimal clinically important diference, *PCS* physical component score, *MCS* mental component score, *NRS* numerical rating scale

global parameters $(p < 0.001)$. Specifically, 3COs had the best impact on T1PA at L2-4, with a 4° correction gain per grade. Pelvic compensation was normalized best at L1 and L2, while L1PA and L4PA were best corrected at L5. Grades 4 and 5 properly distributed lordosis in the lower lumbar spine 50% of the time at all levels except L5. Pelvic non-response and lower extremity compensation (assessed through sacrofemoral, knee, and ankle fexion angles) were relieved more often with increasing 3CO grade. Notably, 3CO patients also had greater correction in all eight lower extremity parameters (all $p < 0.01$).

Cost outcomes

The increased invasiveness, length of stay, and complication rates trended toward a higher perioperative cost for 3CO patients (\$42,806.04 vs. \$40,045.60, $p = 0.086$). However, upon follow-up, 3CO patients had signifcantly higher rates of improvement from preoperative state and would more often to choose to have the surgery again (79% vs. 55%, $p = 0.013$).

Discussion

Adult spinal deformity has been shown to have detrimental impacts on quality of life $[6–11]$ $[6–11]$. As technologies continue to improve, spine surgeons are presented with expanding opportunities to treat patients with increasingly severe frailty and comorbidities [[4](#page-7-9)]. This further drives the development of newer, less invasive techniques which have been shown to improve patient outcomes. However, outcomes in adult spinal deformity have previously been shown to correlate strongly with meeting certain realignment goals [\[14](#page-7-6)]. Thus, there exists a point at which baseline deformity is too severe for these less invasive approaches and techniques.

While standard ASD patients may achieve correction without highly invasive techniques, the complex ASD population represents a unique challenge in which the use of 3CO may be necessitated despite the increased rates of complications [[16\]](#page-7-7).

In this study, patients presented with a high degree and complexity of baseline deformity and, accordingly, underwent 3COs at a higher rate than previous ASD cohorts. Consistent with prior literature, 3CO patients experienced greater perioperative and postoperative complications. Importantly, signifcantly greater radiographic correction in multiple parameters (lumbopelvic, global alignment, lower extremity compensation) was seen. Additionally, increasing grade of 3CO was associated with incrementally greater correction. While 3CO patients did incur higher costs, this was not signifcant compared to No 3CO patients and is potentially offset by greater rates of postoperative improvement reported by 3CO patients. Despite greater rates of complications and potentially higher cost, this study affirms that 3CO represents a powerful and necessary realignment tool for patients with the most severe spinal deformities.

Our study was not without limitations. Of most importance was our decision to utilize Medicare allowable rates for our cost comparison, as previous studies have found signifcant diferences in direct hospital costs compared to Medicare allowable rates in adult spinal deformity surgery [[21](#page-7-15)]. However, we felt Medicare rates would represent a suitable means of standardizing costs across diferent participating centers and improve the generalizability of study fndings [\[17\]](#page-7-8). Similarly, due to recent dispute for carrying the utility gained over two years out to life expectancy in order to calculate QALYs gained for the remaining amount of a patient's life, we did not determine or report the comparison of those values [[24](#page-7-16), [25\]](#page-7-17). Due to the availability of data for all patients at the time of this analysis, we also examined the 6-week outcomes of this cohort, and we plan to further elaborate on the results of our current study with mid-term data once fnalized for this cohort. However, due to the unique aspects of our cohort and the high perioperative risk encountered with three-column osteotomies, we believe these fndings provide a suitable addition to the current literature. Future studies should assess the durability of these realignment strategies in long-term studies to further delineate the attributable beneft of meeting radiographic targets. Although data were collected

prospectively, there is the prospect for a surveillance and classifcation bias that cannot be quantifed. These fndings are meant to be informative for future follow-up studies and trials with strict protocols to investigate factors for improvement of current reimbursement standards and practices. Furthermore, while modeled to the best of our ability, surgical decision-making is challenging to model and there may be aspects of residual confounding that infuence the results of this study, not limited to expertise and selection bias.

Conclusion

Three-column osteotomy usage in contemporary complex spinal deformities is generally limited to more disabled individuals undergoing the most severe sagittal and coronal realignment procedures. While there is a perioperative cost and prolongation with usage, these techniques represent the most powerful realignment techniques available with dramatic impact on normalization at operative levels and reciprocal.

Author contributions The authors made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; drafted the work or revised it critically for important intellectual content; approved the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; Tyler K. Williamson and Peter Passias contributed to conception and design and drafting of the manuscript; Tyler K. Williamson, Jamshaid Mir, Justin Smith, Virginie Lafage, Renaud Lafage, Breton Line, Bassel Diebo, Alan Daniels, Jefrey Gum, D. Kojo Hamilton, Justin Scheer, Robert Eastlack, Andreas Demetriades, Khaled Kebaish, Stephen Lewis, Lawrence Lenke, Richard Hostin, Munish Gupta, Han Jo Kim, Christopher Ames, Douglas Burton, Christopher Shafrey, Eric Klineberg, Shay Bess, and Peter Passias performed acquisition, analysis, and interpretation of data and critical revision of the manuscript for important intellectual content; approved the version to be published; and agree to be accountable for all aspects of the work; Tyler K. Williamson was involved in statistical analysis; Peter Passias was involved in administrative, technical, or material support and did supervision.

Funding The International Spine Study Group (ISSG) is funded through research grants from DePuy Synthes and individual donations.

Data availability The data used in this study is not publicly available, but institution-specifc data may be available upon request from the diferent institutions involved in this study.

Declarations

Conflict of interest None.

Ethical approval Institutional Review Board approval was obtained before enrolling patients in the prospective database. Informed consent was obtained from each patient prior to enrollment.

Consent to participate Informed consent was obtained from all individual participants included in this study.

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