



# Accuracy of pedicle screw insertion by AIRO<sup>®</sup> intraoperative CT in complex spinal deformity assessed by a new classification based on technical complexity of screw insertion

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## Abstract

**Purpose** To develop a classification based on the technical complexity encountered during pedicle screw insertion and to evaluate the performance of AIRO<sup>®</sup> CT navigation system based on this classification, in the clinical scenario of complex spinal deformity.

**Materials and methods** 31 complex spinal deformity correction surgeries were prospectively analyzed for performance of AIRO<sup>®</sup> mobile CT-based navigation system. Pedicles were classified according to complexity of insertion into five types. Analysis was performed to estimate the accuracy of screw placement and time for screw insertion. Breach greater than 2 mm was considered for analysis.

**Results** 452 pedicle screws were inserted (T1–T6: 116; T7–T12: 171; L1–S1: 165). The average Cobb angle was 68.3° (range 60°–104°). We had 242 grade 2 pedicles, 133 grade 3, and 77 grade 4, and 44 pedicles were unfit for pedicle screw insertion. We noted 27 pedicle screw breach (medial: 10; lateral: 16; anterior: 1). Among lateral breach ( $n = 16$ ), ten screws were planned for in–out–in pedicle screw insertion. Among lateral breach ( $n = 16$ ), ten screws were planned for in–out–in pedicle screw insertion. Average screw insertion time was  $1.76 \pm 0.89$  min. After accounting for planned breach, the effective breach rate was 3.8% resulting in 96.2% accuracy for pedicle screw placement.

**Conclusion** This classification helps compare the accuracy of screw insertion in range of conditions by considering the complexity of screw insertion. Considering the clinical scenario of complex pedicle anatomy in spinal deformity AIRO<sup>®</sup> navigation showed an excellent accuracy rate of 96.2%.

**Keywords** Complex spinal deformity · AIRO<sup>®</sup> intraoperative CT · Pedicle classification · Accuracy · Navigation

## Introduction

Insertion of pedicle screws is based on anatomical landmarks which are considerably distorted in the clinical scenario of spinal deformity and revision surgery. Malpositioned pedicle screws can cause significant damage to neurological and vascular structures, and the risk of incorrect screw placement is higher in the deformed spine [1, 2].

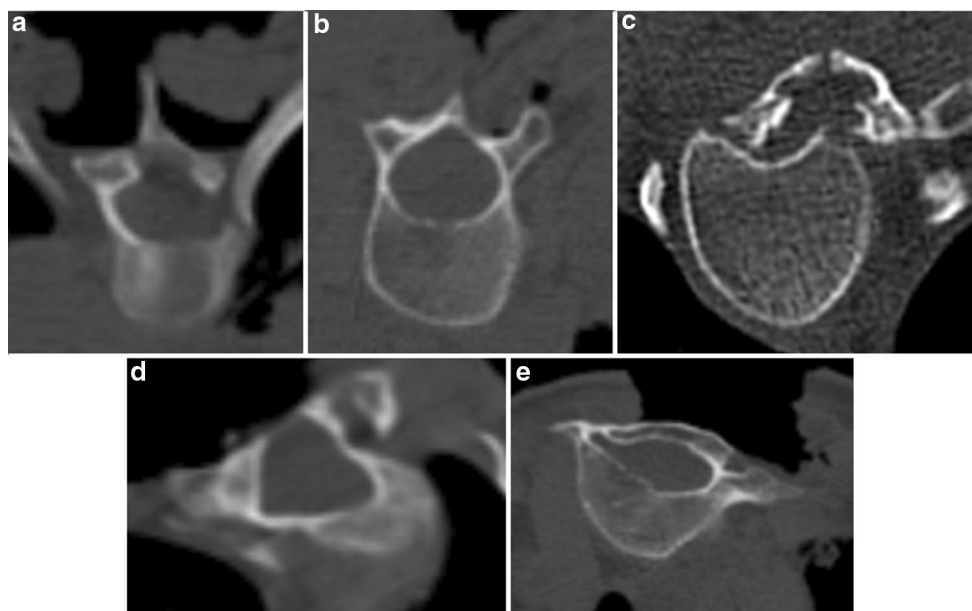
Pedicle morphology differs between idiopathic, neuromuscular, dystrophic, and congenital types of scoliosis. Morphological variants may include thin pedicle, altered

pedicle-body angle, wavy pedicle, hypoplastic pedicle or in a rare case scenario a completely absent pedicle (Fig. 1). Intraoperative recognition of this altered pedicle anatomy is paramount to safety. Different strategies are required for differing morphology and may even end in a decision to abandon a pedicle if morphology precludes safe insertion of screws.

Spine navigation systems have undergone a significant evolution since its inception. First generation navigation systems used pre-operative CT, manual calibration with point-matching registration which was a tedious process [3, 4]. This difficulty was overcome with the advent of the second-generation intraoperative 3D-based navigation system such as Iso-C and O-arm systems [4]. Most recent third generation navigation systems such as the AIRO allow for intraoperative CT with automatic registration.

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**Fig. 1** Morphological variants of pedicle **a** wavy pedicle, **b** very thin waisted pedicle with the isthmus showing no cancellous core, **c** absent pedicle with coronal translation noted between the anterior

vertebral body and the posterior elements, **d** wind-swept pedicle, and **e** asymmetry of body-pedicle angle at same level

While use of navigation for routine pedicle screw insertion is being debated, there is consensus over its clinical utility in improving accuracy rates and decreasing complications in complex spinal deformity [5–10]. The previous studies on accuracy of navigation have not considered the complexity encountered during screw insertion in the deformed spine [11, 12], and this makes comparison between two studies difficult. Hence, it is mandatory that there should be uniformity in the method of assessment of complexity involved in screw insertion in different pathologies when evaluating accuracy of different navigation methods. In addition to cost of AIRO<sup>®</sup> intraoperative computed tomography (iCT) being a concern, it needs evaluation under challenging scenario for cost justification. We propose a classification based on the technical complexity encountered during pedicle screw insertion and evaluated the performance of AIRO<sup>®</sup> CT navigation system based on this classification; in the clinical scenario of complex spinal deformity, curves' magnitudes were in excess of 60°.

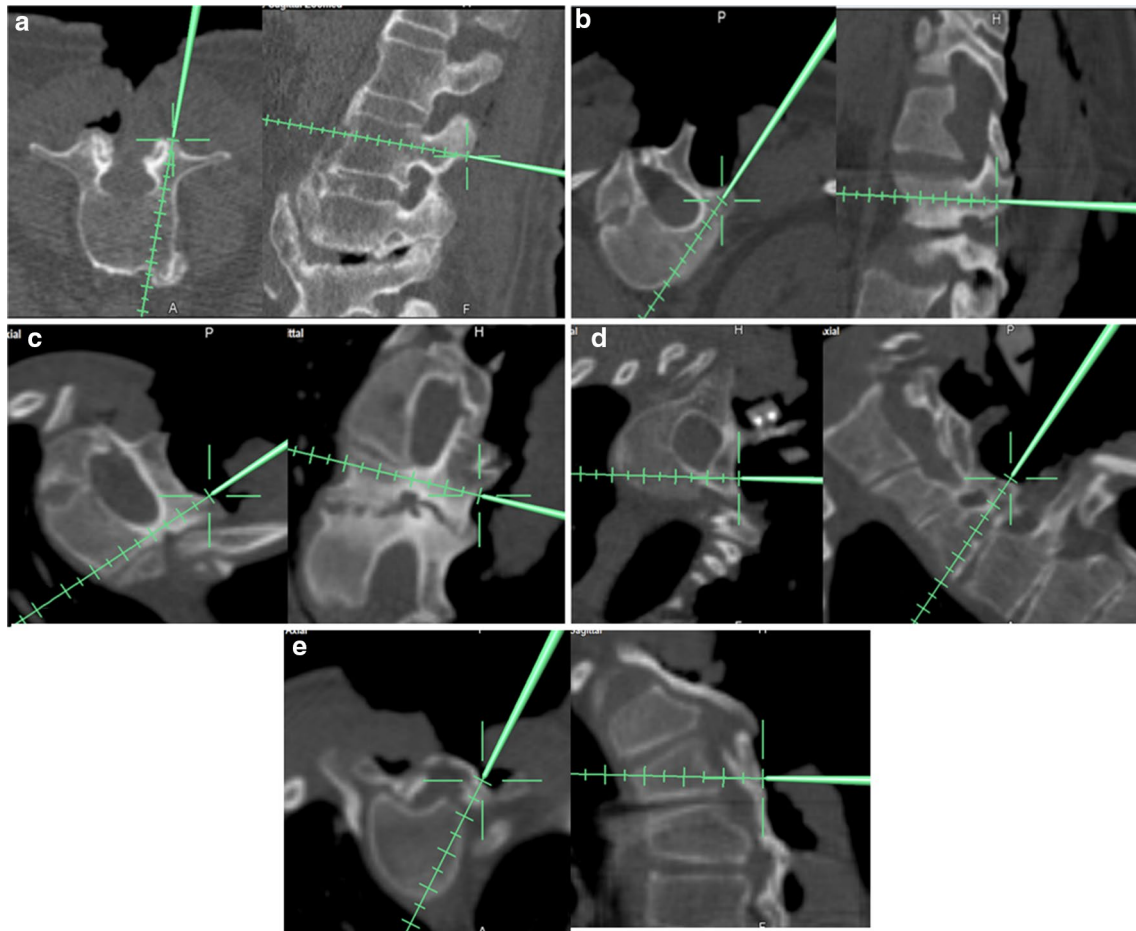
We aimed to analyze the performance of AIRO<sup>®</sup> navigation system for—accuracy and screw insertion time and compared it to the previous navigation systems with regard to portability, scan volume, image quality, and versatility of use in various clinical deformity scenarios.

## Materials and methods

Thirty-one consecutive patients with complex spinal deformity of the thoracic and lumbar spine were prospectively evaluated from January 2016 to April 2016. The study was approved by the institutional review board and informed consent was obtained from all participants. The inclusion criteria were idiopathic scoliosis curve of > 60° deformity, congenital scoliosis, neuromuscular scoliosis, dystrophic scoliosis, and revision deformity surgery.

Pedicles were classified according to increasing technical difficulty of screw insertion into five types (Fig. 2): type 1—normal anatomy, primary surgery, and no deformity, type 2—deformity surgery and revision lumbar surgery, type 3—revision cervical and thoracic surgery, altered pedicle anatomy, and congenital deformity, type 4—pedicle screw insertion unsafe without navigation due to complex trajectory, and type 5—pedicle unfit for screw insertion.

All surgeries were performed by the single surgeon with over 10 year experience in the treatment of deformities by navigation guidance. Posterior approach with pedicle screw instrumentation for deformity correction was performed in all cases. We used the mobile AIRO<sup>®</sup> CT scanner (Brainlab AG, Feldkirchen, Germany) for iCT-based navigation system. For navigation, the AIRO<sup>®</sup> is connected to an image-guidance system and infrared tracking camera (BrainLab CurveTM, Brainlab AG, Feldkirchen, Germany).



**Fig. 2** Pedicle classification based on increasing technical difficulty of screw insertion. **a** Type 1—normal anatomy, primary surgery, and no deformity, **b** type 2—deformity surgery and revision lumbar surgery, **c** type 3—revision cervical and thoracic surgery, altered pedicle

anatomy, and congenital deformity, **d** type 4—pedicle screw insertion unsafe without navigation due to complex trajectory, and **e** type 5—pedicle unfit for screw insertion

## Pedicle screw insertion

After the standard posterior exposure, the minimally invasive reference array was fixed to the spinous process in the bottom of surgical field for  $\leq 6$ -level exposure and in the middle of the surgical field for  $> 6$ -level exposure. The reference array was placed transversely to avoid interference with screw placement. The navigation camera was adjusted to allow co-registration of the minimally invasive reference array and the registration fiducials mounted on the AIRO<sup>®</sup> gantry. All iCT scanning was performed under apnea ventilation following pre-oxygenation to reduce motion artifacts.

Validity of the system was checked by placing a navigated probe on a known anatomic landmark and visually correlating the position to that shown on the image-guidance system and it was repeated intermittently throughout the operation. A pointer tool was used to identify the entry site and develop an appropriate trajectory for the

pedicle screw. The pedicle pathway was gently created using a sharp pedicle finder or with a drill. The direction and depth of prepared tract were confirmed frequently using the tool navigator. The length and diameter of the screw were selected based on the virtual screw dimensions superimposed on the images of the pedicle. In thinned, deformed, and sclerosed pedicles, where transpedicular screw fixation was impossible, an in-out-in technique with a planned lateral pedicle wall breach was performed under navigation guidance. To prevent loss of fidelity, facetectomies, osteotomies, and other procedures to improve curve flexibility were performed after screw insertion.

Following the instrumentation, all screws were evaluated by a second iCT. Screws were analyzed for breach of the pedicle wall—medially, laterally, inferiorly, and superiorly. Any breach of the anterior vertebral cortex was measured on the axial sections. Screw placement was assessed based on classification, as shown in Table 1. Breach greater than  $< 2$  mm was excluded from the analysis, as these were

**Table 1** Grading used for pedicle breaches on intraoperative CT scan

Category	Grade	Description
Assessment of screw position	0	No breach
	1	Only the threads outside the pedicle; less than 2 mm
	2	Core screw diameter outside the pedicle; breach 2–4 mm (non-critical breach)
	3	Complete screw outside the pedicle (critical breach)

likely to be insignificant or “silent” breaches. “Biomechanically critical breaches” were considered as follows—non-critical breach 2–4 mm and critical breach > 4 mm. Any critical breach with medial perforation was revised. We also calculated screw insertion time from the placement of tool navigator to the completion of screw placement.

For each case, we recorded the total radiation exposure from intraoperative CT in mGy/cm (Dose Length Product, DLP). Then, radiation exposure was converted to effective dose (E) (in milliSievert, mSv). Effective doses for intraoperative CT were calculated using E-to-DLP ratio reported by the National Council of Radiation Protection Report 160 [13] and the American Association of Physicists in Medicine Report 96 [14].

## Results

The study group consisted of 31 patients with deformity meeting the inclusion criteria. Twenty-four patients had scoliotic deformity (idiopathic 12, congenital 6, and neuromuscular 6) and seven patients had kyphotic deformity (congenital 6 and Scheuermann’s kyphosis 1). The average age of the patients was 14.3 years (range 11–24) and the average Cobb angle was 68.3° (range 60°–104°). The average number of instrumented segments was 12 (range 5–15) and mean number of screws per patient was 15 (range 7–24).

A total of 452 pedicle screws were inserted. Screws were placed from T1 to sacrum. There were 116 pedicle screws in the upper thoracic spine, 171 in the lower thoracic spine, and rest 165 in lumbo-sacral spine. Average blood loss was 847 ml (range 250–1400).

There were total of 27 screws with a *biomechanically critical pedicle breach* (2–4 mm and > 4 mm breach) noted (Table 2), including 10 medial, 16 lateral wall breaches, and 1 anterior body penetration. No patient was clinically symptomatic for malpositioned pedicle screw. There were 39 screws with < 2 mm breach which may be considered “silent” or insignificant and were excluded from the accuracy analysis.

Analyzing the *biomechanically significant breaches* further, among medial breach ( $n = 10$ ), only one screw had a critical breach (> 4 mm), needing reinsertion during index procedure, and nine screws had non-critical breach

**Table 2** Radiological assessment of pedicle screw placement

Total no. of screws	452
Medial pedicle breach (mm)	
< 2	19
2–4	9
> 4	1
Lateral pedicle breach (mm)	
< 2	15
2–4	14 (planned-10)
> 4	2
Superior pedicle breach (mm)	
< 2	1
2–4	0
> 4	0
Inferior pedicle breach (mm)	
< 2	2
2–4	0
> 4	0
Anterior body penetration (mm)	
< 2	2
2–4	1
> 4	0

(2–4 mm). Among lateral breach ( $n = 16$ ), ten screws were planned for in–out–in pedicle screw insertion, and four screws showed non-critical breach (2–4 mm) and two screws with > 4 mm breach. After accounting for planned breach, the effective breach rate was 17/452 screws (3.8%) with an accuracy rate of 96.2%. We also encountered 44 pedicles which had to be abandoned, as pedicle screw insertion was not possible.

Based on configuration of pedicle anatomy, we had 242 type 2 pedicles, 133 type 3 pedicles, and 77 type 4 pedicles (Table 3). We did not have any type 1 pedicles, as all cases had spinal deformity. Forty-four pedicles were classified as type 5 pedicles, where it was completely absent or severely wasted, too rotated, and trajectory not possible. Assessment of pedicle breaches based on new classification showed 4.13% breach in type 2 pedicles, 9.02% pedicle breach in type 3 pedicles, and 6.49% breach in type 4 pedicles (Table 3).

**Table 3** Pedicle screw breaches based on new pedicle classification

Pedicle types	Medial grade I <sup>a</sup>	Medial grade 2	Medial grade 3	Lateral grade I <sup>a</sup>	Lateral grade 2	Lateral grade 3	Superior grade I <sup>a</sup>	Inferior grade I <sup>a</sup>	Anterior body penetration grade I <sup>a</sup>	Anterior body penetration grade 2	Breach/no. of pedicles	Breach rate (%)
1												
2	11	6	7	3	1		1				10/242	4.13
3	5	3	5	9		1	1	1			12/133	9.02
4	3		3	2	1			1		1	5/77	6.49
5											44 <sup>b</sup>	

<sup>a</sup>All grade 1 breaches were excluded from the final analysis, as placement was considered accurate accounting for scatter effect seen on CT scan

<sup>b</sup>No screws were inserted for 44 pedicles

In all cases, we were able to scan whole of the planned instrumented levels in one single scan. We did not have any difficulty in visualization of vertebrae because of magnitude of kyphoscoliotic deformity. Average screw insertion time was  $1.76 \pm 0.89$  min (range 0.42–5.35 min). Average radiation exposure to the patient was  $4.85 \pm 1.18$  mSv (range 2.24–9.42 mSv). The operating room personnel and surgeons stayed outside the operating room during scan.

### Discussion

Pedicle screw constructs offer many advantages over hook and wire constructs in treatment of spinal deformity and is considered by many to be the method of choice for deformity correction [15, 16]. The accurate placement of pedicle screws is critical for optimal biomechanical hold, successful deformity correction and avoiding damage to the neural elements, major vessels, and viscera in close vicinity of the spine [2]. Spinal navigation allows for real-time images and three-dimensional anatomic reconstruction of the deformed spine, thus offering an option of screw insertion in complex pedicle morphology and orientation [17].

Computer-assisted navigation has helped improve the accuracy of screw placement. Rajasekaran et al. quoted accuracy rate of 97% in comparison with 61% in free-hand technique [18]. Merloz et al. noted that screw placement with navigation compared to fluoroscopy guidance had lower rates of misplacement 5% compared to 14.6% [19]. Literature review puts accuracy rates for CT-navigated pedicle screws ranging from 89 to 100% [6].

The previous studies have compared various navigation systems over the process of evolution from virtual fluoroscopy-guided navigation to the current state of art AIRO<sup>®</sup> iCT navigation system. For comparing accuracy of various navigation technologies or for comparing two different series, it is important that there is a pedicle classification that grades the technical difficulty encountered during screw insertion. A pedicle classification based on core diameter for assessment of accuracy is over simplistic and is not suitable, especially in the clinical scenario of complex spinal deformity. Pedicles can vary significantly based on the nature of pathology, etiology of deformity, region, and level of deformity. In spinal deformity, apart from pedicle diameter, pedicle orientation, morphology, and trajectory of screw can create additional complexity during insertion. The pedicles often assume a “wind-swept” appearance in larger curves when the spine rotates toward the convexity of the scoliosis [20]. In dystrophic scoliosis severe rotation of the apical vertebra, scalloped vertebral body margins with widened spinal canal can result in severely waisted pedicles [21, 22], which are very difficult to instrument. Similarly, in congenital scoliosis abnormal pedicle size, fused posterior



hemi-lamina, and aberrant pedicle orientation makes screw insertion technically demanding. Watanabe et al. classified pedicles based on pedicle channel osseous anatomy into large cancellous channel, small cancellous channel, cortical channel, and slit/absent channel [11]. Similarly, Zhang et al. classified pedicle based on inner cortical width [12]. However, they did not consider spatial orientation of pedicles, which holds a great significance especially in severe deformity. Therefore, we incorporated three-dimensional orientation of pedicle as well as adjacent bony landmarks while classifying them in our classification.

Our proposed classification includes normal pedicles under type I, where cost effectiveness of use of navigation may be a concern. Our series included only complex deformity cases, so we had no type 1 pedicles. Complexity of cases was evident from the fact that 46% of pedicles were categorized as types 3 and 4. We also confronted with 17% of pedicles, where screw insertion without navigation was unsafe due to complex trajectory. In our series, navigation also prevented attempts at instrumentation, where pedicles were absent or much attenuated. We encountered 44 pedicles which were unfit for screw insertion based on the navigation finding. In complex deformity, often, the vertebral body anteriorly and the lamina posteriorly are translated in a position, such that a routine screw entry point would result in a direct canal violation with disastrous consequences. Therefore, our classification emphasizes the role of altered posterior surface landmarks, pedicle anatomy as well as three-dimensional orientation. Usage of this classification helped us to analyze the utility of AIRO® in complex spinal deformity. Considering the cost of AIRO® and other such navigation systems, their true utility is best assessed for pedicle grades 3–5.

Spinal navigation procedure has evolved over time from 2D virtual fluoroscopy to the latest in the armamentarium, Mobile AIRO® intraoperative CT. This 32-slice CT scanner offers an extra-large gantry opening of 107 cm while at the same time possesses an extra small footprint of 1.5 m<sup>2</sup>, offering excellent portability. AIRO® offers the imaging

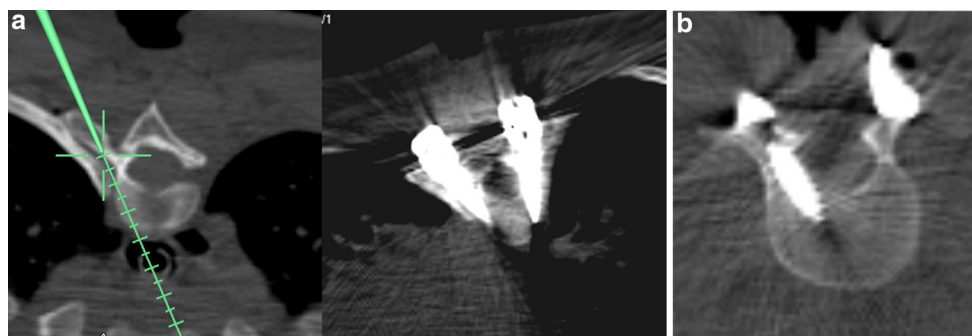
quality of a diagnostic CT scanner, with a scan volume of 51.2 cm × 100 cm. It does not suffer from necessity of iso-centricity. It can scan the whole spine in 45 s.

In 2014, Hecht et al. conducted a study on accuracy and workflow of navigated spinal instrumentation with the mobile AIRO® CT scanner on 23 subjects with degenerative disease, trauma, and tumor [23]. Their analysis of screw placement accuracy revealed accuracy rate of 95.9%.

Jin et al. studied accuracy of screw placement near apical region in 32 patients with dystrophic neurofibromatosis-1-associated scoliosis [24]. They reported accuracy rate of 79% using O-arm CT navigation as compared to free-hand technique with accuracy rate of 67%. Liu et al. reported 91.7 and 93.8% satisfactory screw insertion within small ( $\leq 3$  mm) and large ( $> 3$  mm) thoracic pedicles, respectively, using O-arm navigation system [25].

Screw penetration across the pedicle boundaries is used to evaluate the accuracy of navigation systems. The quantitative penetration in the previous reports has been divided into three grades as follows (grade 1:  $< 2$  mm, grade 2: 2–4 mm, and grade 3:  $> 4$  mm) [16, 21, 22, 24, 25]. Grade 1 breaches have been considered insignificant by other authors when assessing accuracy of pedicle screw placement by free-hand or image-guided techniques [16, 21, 22, 25].

In this study, pedicle breaches  $< 2$  mm penetration was considered insignificant due to the scatter effect seen on CT scans, even with the use of thin slice CT scans and titanium implants. Deciphering a breach  $< 2$  mm would be difficult with such scatter artifacts seen on CT images, and frequently, such penetrations are asymptomatic for the malpositioned implant. For these reasons, breaches 2–4 and  $> 4$  mm were considered significant and included in the final assessment of accuracy rate. In addition, frequently, the dimensions of the deformed pedicles would preclude the placement of an entirely contained screw even with the smallest available screws. Among 16 lateral breach, 10 screws were planned for in–out–in pedicle screw insertion (Fig. 3). These pedicles were thinned, deformed, and sclerosed, where transpedicular screw fixation was impossible, so an in–out–in



**Fig. 3** Pedicle breaches **a** planned in–out–in lateral pedicle breach in thin-sclerosed pedicle and **b** unplanned medial grade 2 pedicle breach

technique was used under navigation guidance. We did not revise screws with medial breach < 4 mm.

Gertzbein and Robbins [26] reported a safe zone of 4 mm (2 mm epidural space and 2 mm subarachnoid space) for pedicle screw passage as well as Kim et al. [27] quoted 2–4 mm of cortical breach as safe encroachment. Based on these observations, we revised only critical breaches > 4 mm. No case was clinically symptomatic for malpositioned pedicle screw in the postoperative period. In certain instances, with pedicle diameter  $\leq$  3 mm, breach was unavoidable as we used wider diameter screws in these pedicles to get a better pull-out strength. Navigation allowed us to assess diameter and length of screws that can be negotiated through the pedicle based on projected measures on the navigation screen.

Cui et al. [28] reported average screw insertion time of 3.48 min in the navigation group, while Rajasekaran et al. [18] clocked screw insertion time (including average data acquisition time per screw) in the navigation group  $2.37 \pm 0.72$  min (range 1.16–4.5) per screw. Our study reported average screw insertion time of  $1.76 \pm 0.89$  min (range 0.42–5.35 min). Probable reason for the short screw insertion time was significant experience of the first author in the technique of navigation-based surgery and associated learning curve of the technique.

Although intraoperative CT systems spare the operative team from scattered radiation associated with fluoroscopy, there is the potential for increased dosing to the patient with intraoperative CT navigation depending on the settings on the intraoperative CT. To minimize the radiation exposure, we used imaging protocols on the basis of body mass index. Our study showed average radiation dose per scan as  $4.85 \pm 1.18$  mSv (range 2.24–9.42 mSv); similarly, Liu et al. reported radiation dose of one intraoperative 3D scan using O-arm as approximately 4.2 mSv (range 2.8–7.1 mSv) [25].

Su et al. have reported on the comparison of fixed low-dose pediatric radiation protocol versus manufacturer-recommended protocol in the use of the O-arm-based navigation [29]. They noted that fixed low-dose radiation protocols gave adequate intraoperative O-arm images, and offered four times lower radiation dose compared to the mean annual background radiation and ten times lower compared to manufacturer-recommended default protocols. The authors concluded that such low-dose protocols can provide adequate image quality and offer higher index of radiation safety for pediatric patients.

The AIRO system automatically calculates the appropriate CT scan protocols based on body weight using in-built system recommendations. The AIRO navigation system offers radiation dose reduction in the form of three options of resolution at the time of scan protocol selection 25, 50, and 75%. We selected 75% resolution for our pediatric patients. The mean radiation dose with such a selection though was

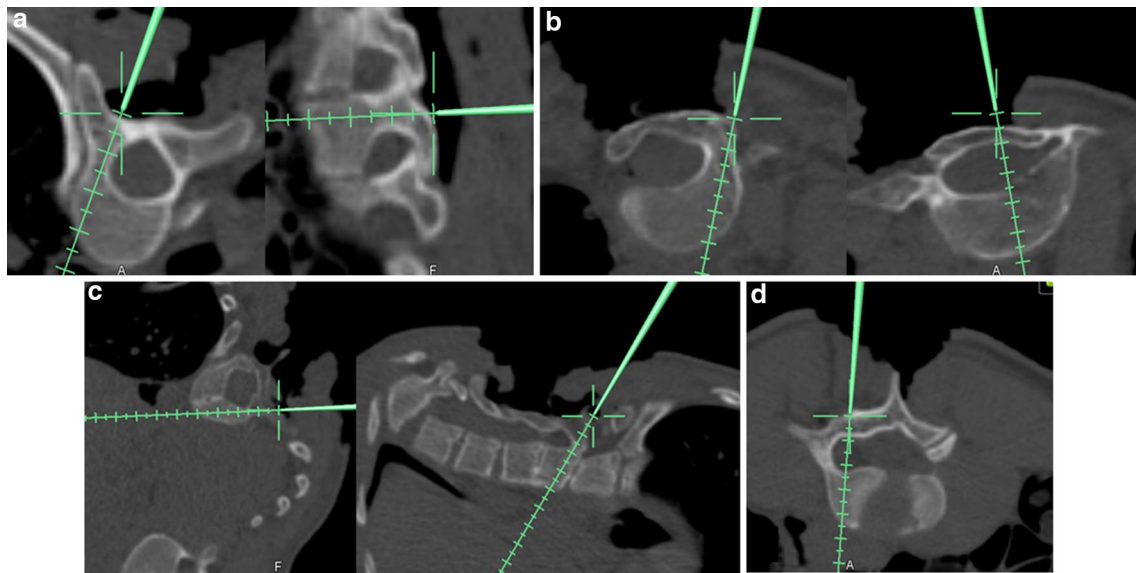
still higher than values reported by Liu et al. [25] and Su et al. [29].

Putzier et al. reported on 4 patients with 76 screws, where pre-operative CT scan-based 3D printing was used to develop a positioning guide and to decide accurate trajectory of screw placement in scoliosis. The authors suggested that such 3D-printing developed models may be used as an alternative to intraoperative navigation systems, where initial cost and maintenance of conventional navigation systems are not feasible [30].

Pireau et al. reported excellent accuracy (96.1%) with cone beam CT-based navigation system used to insert screws in thoracic and lumbar pedicle screw for degenerative, tumor, and traumatic conditions. The images provided with a low-dose radiation protocol were found satisfactory and showed excellent accuracy. The authors excluded scoliosis, as cone beam CT could scan only a maximum of five levels; thus, it would need multiple scans in scoliosis surgery [31]. Recently, total navigation systems are available which include navigated burrs, bone drills, taps, and screws.

Navigation showed special utility in multiple scenarios (Fig. 4). It helped us to recognize complex pedicle trajectory in severely rotated and deformed pedicles. AIRO<sup>®</sup> demonstrated a dramatic difference between concave and convex side pedicles in terms of trajectory, dimensions, and orientation. The significant difference in pedicle anatomy at adjacent levels was identified in this series. Malpositioned pedicle screws were prevented in widely placed pedicles. It allowed screw placement in pedicles too thin for even smallest screw available and helped in the identification of regions of the deformity with absent pedicles. In congenital scoliosis, navigation helped us to characterize the anatomy of hemivertebra, define the osteotomy margins as well as real-time guidance during the procedure as well.

CT navigation allows a real-time view of the pedicle and probe trajectory. It also allows for small alterations in the starting point and screw trajectory to determine the optimum tract for the navigated probe. In many instances, the trajectory selected using intraoperative CT navigation guidance is quite different, which would be anticipated from the standard surface landmarks and pre-operative templating. Screw placement should occur immediately after the registration scan without further decompression, facetectomy, or discectomy. Segmental motion allows for a potential slight shift of vertebral segments with respect to each other during bone and disc space work, which may lead to diminished screw accuracy and result in pedicle breaches. Using such precautions AIRO<sup>®</sup> iCT navigation can give excellent result even in complex deformity scenarios.



**Fig. 4** Special utility of AIRO® iCT navigation in different scenarios, **a** dramatic difference between concave and convex side pedicles of T7 vertebra in terms of trajectory, dimensions, and orientation, **b** vastly different pedicle dimensions at adjacent levels seen with; right L3 pedicle showing wide pedicle diameter, while right L4 pedicle showing altered body-pedicle angle, and **c** showing a significant

buckling collapse with scoliotic deformity. Though the patient is lying prone, the deformed T8 pedicle appears to be nearly horizontally oriented. Thus, the screw insertion trajectory was significantly contorted. **d** Routine starting point at the left L3 pedicle likely to result in intra-canal pedicle screw placement

## Conclusion

Studies comparing accuracy of different forms of navigation have an inherent flaw; in that, the technical difficulty of screw placement, depending on the disease pathology and pedicle morphology, is not standardized. To overcome this deficiency in the literature, we have devised a classification with five types of increasingly complex pedicle screw insertion scenarios. This will allow for comparison on studies using different navigation-based technologies and results from various institutions. The study also assessed accuracy of AIRO navigation systems, where the study population was limited to technically challenging pedicles for screw placement. We found an accuracy of 96.2% and found AIRO to be safe and useful in complex spinal deformity.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical statement** The study was approved by the institute review board and informed consent was obtained from all participants.

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