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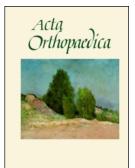
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### Stability and migration across femoral varus derotation osteotomies in children with neuromuscular disorders

#### 1-year RSA results

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Background and purpose — Studies have indicated that one-third of children with cerebral palsy (CP) develop dislocation of the hip that needs surgical intervention. When hip dislocation occurs during childhood surgical treatment consists of tenotomies, femoral varus derotation osteotomy (VDRO), and acetabuloplasty. Relapse is observed in one-fifth of cases during adolescence. In this prospective cohort study, we performed a descriptive evaluation of translation and rotation across VDROs in children with neuromuscular disorders and syndromes by radiostereometric analysis (RSA). We assessed "RSA stability" and migration across the VDROs.

Patients and methods — Children with a neuromuscular disorder were set up for skeletal corrective surgery of the hip. RSA follow-ups were performed postoperatively, at 5 weeks, and 3, 6, and 12 months after surgery.

Results — 27 femoral VDROs were included; 2 patients were excluded during the study period. RSA data showed stability across the VDRO in the majority of cases within the first 5 weeks. At the 1-year follow-up, the mean translations (SD) of the femoral shaft distal to the VDRO were 0.51 (1.12) mm medial, 0.69 (1.61) mm superior, and 0.21 (1.28) mm posterior. The mean rotations were 0.39° (2.90) anterior tilt, 0.02° (3.07) internal rotation, and 2.17° (2.29) varus angulation.

**Interpretation** — The migration stagnates within the first 5 weeks, indicating stability across the VDRO in most patients.

Cerebral palsy (CP) is a multidimensional neurological disease that begins before birth or in early childhood and persists throughout life (Minciu 2012). The incidence of CP in Denmark is 2 in 1,000 live births and the incidence has been stable since the 1990s (Ravn et al. 2010). Common symptoms in the

extremities are spasticity and rigidity, which lead to reduced mobility (Minciu 2012). The motor function is often classified with the gross motor function classification system (GMFCS), which indicates the severity of the CP (Palisano et al. 1997).

Hip dislocation in children with CP is a common complication, and has been observed in 28–35% of cases, with a high positive correlation to the classification of severity by GMFCS (Samilson et al. 1972, Soo et al. 2006). Hip dislocation causes severe pain in the long term, since there is high risk of developing secondary hip arthrosis (Cooperman et al. 1987, Soo et al. 2006). The degree of hip dislocation is measured on an AP pelvic radiograph using the femoral head extrusion index (FHEI), where the percentage of uncovered femoral head is measured (Heyman and Herndon 1950, Reimers 1980).

Treatment is surgical "relocation" of the hip by combined procedures of adductor and psoas tenotomies, femoral varus derotation osteotomy (VDRO), and acetabuloplasty (Canavese et al. 2010, Dhawale et al. 2013) (Figure 1). Despite surgical intervention, relapse is observed in 16–25% of cases, and postoperative progressive hip dislocation is believed to be due to the continuous effect of CP, skeletal growth, and remodeling. In rare cases the relapse is caused by malunion, non-union, or pseudoarthrosis (Samilson et al. 1972, Bennet et al. 1982, Dhawale et al. 2013). A recent study showed that up to 56% of children with severe CP who undergo surgical relocation of the hip have unsatisfactory results in the long term (Canavese et al. 2010).

Radiostereometric analysis (RSA) is a high-precision method and has the ability to identify complex 3D migration of skeletal structures. Only a few orthopedic RSA studies in children (Lauge-Pedersen et al. 2006, Gunderson et al. 2013, Horn et al. 2013, Lauge-Pedersen and Hägglund 2013) have been published in the last 2 decades. None of these have

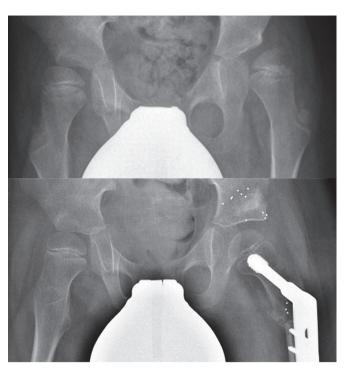


Figure 1. A 7-year-old boy with severe left hip lateralization and acetabular dysplasia preoperative and 6-month postoperatively.

evaluated the stability across osteotomies. A recent RSA study evaluated stability across high tibial osteotomies in adults, and showed limited micromotion and good stability 6–12 weeks after surgery (Teeter et al. 2015).

The main aim of this study was to assess whether initial stability across VDROs performed on children with neuro-muscular disorders and syndromes is detectable by RSA 5 weeks postoperatively. A secondary aim was to estimate the migration at 1-year follow-up of the femoral shaft distal to the VDRO in stable osteotomies, by our definition of RSA stability of an osteotomy. This estimation might be used for early detection of relapse in future studies.

#### Patients and methods

This was a prospective cohort study with trial period from November 1st, 2012 to October 31st, 2014. The patients had a neuromuscular disorder or syndrome and were aged between 2 and 18 years. In all cases, corrective skeletal hip surgery was indicated and planned. The indication for surgery was FHEI > 50%. The only primary exclusion criterion was a revision surgery.

The follow-up period was 1 year, and the RSA examinations were performed postoperatively, after 5 weeks, and at 3, 6, and 12 months. The visit window for the RSA recording was 3 days at postoperative follow-up, 1 week at the 5-week follow-up, and 2 weeks at subsequent follow-ups. No more than 3

attempts were used to obtain acceptable RSA radiographs, to minimize the exposure to radiation.

#### **Ethics**

This study was approved by the local Danish Research Ethical Committee (entry no. H-2-2011-124), and patients were included according to Ethical Committee guidelines.

#### Surgical procedures

In general anesthesia, hips were tested for limitations in range of motion (ROM), and appropriate tenotomies were performed, typically in iliopsoas and the adductor muscles. Afterwards, the varus derotation osteotomy was initiated with a lateral skin incision and a K-wire guide was centralized in the femoral neck. Prior to placing the femoral neck screw, 4-8 tantalum markers (1 mm diameter; Wennbergs Finmek AB, Gunnilse, Sweden) were inserted mainly on the medial side of the screw. Then we performed a transverse osteotomy approximately 1 cm below the lesser trochanter. K-wires were placed on each side of the osteotomy before cutting the proximal femoral shaft; these were used to limit the rotation. 4-8 tantalum markers were placed through the osteotomy down the diaphysis of the femur—alternately in anterior and posterior directions, with the markers as far distal as possible. Afterwards, the varisation and derotation was secured by plate-fixation (Compression Hip Screw; Smith and Nephew, London, UK). An important procedure to obtain optimal RSA radiographs was to estimate the best rotation of hip according to the patella position with most markers visible under fluoroscopy. All patients then had an acetabuloplasty according to Dega, Salter, or Chiari. Still under anesthesia, the hips were immobilized in a spica cast (Scotchcast; 3M) for 5 weeks. After the 5 weeks, the cast was removed and the patient was mobilized without restriction, in collaberation with the physiotherapy unit.

#### RSA methodology

The patients were placed in supine position with the pelvis just above the wheel-borne carbon fiber enclosed uniplanar calibration cage (LUMC, Leiden, the Netherlands) (85 × 29 × 55 cm) in a standardized manner, rotating the hip according to patella when RSA was recorded. 2 ceiled-attached radiographic tubes (Arcoma AB, Växjö, Sweden) were adjusted to the 2 digital detector plates (DRX-1C type; Carestream, Rochester, NY) and angled 46° with a height of 160 cm from the plates. The exposure was standardized to 65 kV and 12.5 mAs. The RSA outcomes were of spatial resolution size 2,560 × 3,072 (gray-sca le, 8-bit dicom format).

The orthogonal directions and the coordinate system used across the VDRO are illustrated in Figure 2. The femoral neck was used as reference position and migration was measured with the femoral shaft distal of the VDRO as the model. The migration is described as follows. Translations: transverse(x+) = medial, longitudinal(y+) = superior, and sagittal(z+) = anterior; and rotations: Rx+ = anterior tilt, Ry+ = internal rotation,

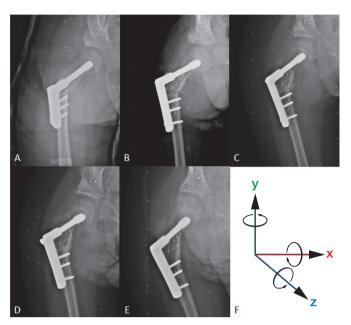


Figure 2. RSA radiographs at postoperative time 0 weeks (A), 5 weeks (B), 3 months (C), 6 months (D), and 12 months (E). In panels D and E, the VDRO is considered radiographically stable. Panel F shows the orientation of the 6 degrees of freedom with positive values for a right distal femur. Hence, translation x + = medial, y + = superior and z + = anterior; and rotation Rx + = anterior tilt, Ry + = internal rotation, and Rz + = varisation.

and Rz+= varisation. Rx, Ry, and Rz values for the left femoral shaft were multiplied with -1 to give the same directions as for the right femur.

The radiographs were analyzed with MbRSA 3.41 software (LUMC, Leiden, the Netherlands). MbRSA software assesses translation and rotation according to the RSA community guidelines defined by Valstar et al. (2005). We also included the screw tips in the femoral shaft as additional markers if too many of the tantalum markers were covered by osteosynthesis material. The 95% repeatability limits (RPLs) of the screw tips and markers were estimated by double examination at 6-month or 1-year follow-up if radiographs indicated healing across the osteotomy (internal RPL). RPL was calculated as the 95% deviation from the true value (Ranstam et al. 2000):

$$RPL_{df} = 1.96 \times \sqrt{2} \times SD_{df}$$

Based on the internal RPL, we defined an RSA-stable VDRO as observation of 2 consecutive follow-ups with migration below the internal RPL for each of the 6 orientations. In addition, we elaborated on the definition if altering the definition RSA stability to stable migration below the RPL in only 5 out of 6 orientations. Before the start of the study, the RSA setup at Hvidovre Hospital was validated on a phantom model. RPLs of translations were 0.06–0.22 mm and RPLs of rotations were 0.19–0.52°. Median maximum total point motion (MTPM) was 0.16 mm (0.07–0.59).

Marker stability was assessed from the mean error of rigid-body fitting (ME), and if the standard threshold of the ME was below 0.35 mm (Valstar et al. 2005). However, we expected remodeling and also growth of the children in the study period, so evaluation of ME individually was protocolled; RSA data were included if the initial ME observation was below 0.35 mm but the subsequent observations were allowed to continuously increase in a manner that could be interpreted as bone remodeling or growth.

The marker distribution was assessed from the condition number (CN). The RSA ISO standards state that CN should be below 120 mm<sup>-1</sup> when studying adult hip, knee, or shoulder arthroplasties, but it may be higher when studying other smaller structures. As in other studies, we included observations if CN < 150 mm<sup>-1</sup> (Söderkvist and Wedin 1993, Onsten et al. 2001, Börlin et al. 2002, Bragdon et al. 2004, Valstar et al. 2005, Gunderson et al. 2010, Fong et al. 2011).

MTPM values are an attempt to express the total translation and rotation as one single value (in mm) (Ryd 1986). The MTPM values are therefore absolute and are possibly not normally distributed, so the results are given as a median (range) (Valstar et al. 2005). MTPM was used as a surrogate measure for ongoing bone remodeling or growth. We planned to test the data by linear regression analysis in cases where the residuals proved to be normally distributed.

#### Results

22 patients were originally included, in whom 27 femoral VDROs were performed. RSA data from 2 patients were excluded from the study. 1 patient died 2 days after surgery due to respiratory distress and 1 patient suffered from collapse of the osteosynthesis material, which was discovered at the 5-week RSA follow-up. Thus, there were 25 femoral VDROs in 20 patients (14 left and 11 right) with a 1-year RSA follow-up period. There were no dropouts during the study period. All of the patients included also had acetabuloplasties performed with insertion of tantalum markers (manuscript submitted). 5 patients underwent bilateral surgery: 1 in 1 session and 3 had their second operation within 1 month of the first surgery. 1 patient waited 6 months for the second surgery. 2 patients missed their 3-month follow-up; otherwise all RSA recordings were obtained.

#### Demography

The cohort consisted of 10 boys and 10 girls with a median age of 9.4 (3.4–16.8) years and 8.9 (3.0–13.7) years, respectively. 10 patients suffered from spastic tetraplegia, 7 had spastic diplegia, 2 had hypotonic tetraplegia, and 1 was dyskinetic. The primary neuromuscular diagnosis was cerebral palsy (n = 17) and the remaining diagnoses were Bohring-Opitz syndrome (n = 1), Rett syndrome, (n = 1), and Angelman syndrome (n = 1).

Table 1. Median femoral head extrusion index (FHEI) and neck-shaft angle (NSA) evaluated on AP pelvic radiographs preoperatively and post-operatively, and the difference between these 2 measurements (n = 18)

		IEI (%) n range	-	ISA range
Preop. Postop. Diff.	54 11 44	(42 to 78) (-23 to 35) (12 to 92)	120°	(145–178) (105–146) (29–60)

All patients had severe disabilities (without ambulation or with ambulation using assistive devices). They were classified according to the GFMCS score for cerebral palsy (Graham 2005). This was applicable to all, even though they had a variety of diagnoses. The distribution was GMFCS-3 (n = 4), GMFCS-4 (n = 5), and GMFCS-5 (n = 11). 14 of the 20 patients had some degree of mental retardation and 10 of these suffered from epilepsy. The main comorbidity was obstipation, for which 10 of the patients received daily medication. The patients were also medicated for sleeping disorders and/or respiratory problems. The median number of daily medical products was 2 (0–6).

#### Pre-, per-, and postoperative observations

The median height was 126 (90–166) cm, median weight was 25 (13–70) kg, and median BMI was 17 (15–27) at the time of surgery. ASA classification was assessed by experienced anaesthesiologists, prior to sedation, to be 2 ASA-1, 10 ASA-2, and 8 ASA-3.

The derotation and varisation were estimated by the surgeon during surgery: median 30° (0-45) of external rotation and 30° (20-40) varisation. 10 of the 26 hips also had shortening femur osteotomy ranging from 1.5 to 2.0 cm. The median duration of surgery was 247 (97–431) min, which also included approximately 1 hour of casting. Median estimated perioperative blood loss was 290 (50-1,700) mL. 4 senior pediatric orthopedic surgeons were responsible for all operations, and none of the operations had perioperative complications. The median casting period was 4.9 (0–6.9) weeks and postoperatively 4 GMFCS-5 patients were treated without cast and not permitted mobilization for 5 weeks (this was also the case in the excluded patient with osteosynthesis failure). In 7 of the 25 surgeries, postoperative complications occurred during the 1-year follow-up. 2 patients suffered from superficial skin infections, which were treated successfully with peroral antibiotics. 2 patients had femoral shaft fractures on the ipsilateral side (after 10-12 weeks; both during physiotherapy). Both patients continued in the study, as the fractures were far distal to the markers. 2 patients had asymptomatic heterotopic ossification (Brooker classification 2).

Table 2. The 95% repeatability limits (RPLs) based on double examination at 10 follow-ups of 10 patients after radiographically stable VDRO, with inclusion of screw tips in the femoral shaft as additional markers

Translation	RPL	Rotation	RPL
x y z	0.23 mm 0.19 mm 0.46 mm	Rx Ry Rz	0.81° 1.24° 0.58°

#### Evaluation of conventional AP radiographs (Table 1)

The median preoperative and postoperative FHEI values were 54% (42–78) and 11% (23–35), respectively. The median reduction of extrusion was  $\Delta$ FHEI 44% (12–92). The median pre- and postoperative neck-shaft angle (NSA) (Hoaglund and Low 1980)) values were 164° (145–178) and 120° (105–145), respectively. Median angle reduction ( $\Delta$ NSA) was 45° (29–60).

#### Results of the RSA

Despite using a standardized setup, the hip spica cast restricted mobility of the operated leg and thus the region of interest—and as predicted, many of the tantalum markers were covered or partly covered by the osteosynthesis material in the femoral shaft distal to the VDRO in the postoperative RSA. RSA data were initially only valid in 8 of the 25 femoral VDROs. If we used a different follow-up RSA as reference model without a hip spica cast in all patients, 19 RSA datasets would be valid, indicating difficulties with the postoperative RSA. The data that were excluded and invalid were (1) from 5 patients due to loose markers (ME > 0.35 mm), and (2) from 1 patient with an insufficient distribution of markers (CN > 150 mm<sup>-1</sup>).

As protocolled, we then included the tip of the screws in the femoral shaft as additional markers and this increased the number of valid datasets to 19 hips from 17 patients, corresponding to 95 follow-ups ( $19 \times 5$ ). 10 follow-ups were subsequently excluded: 2 because of having a CN of > 150 mm-1, 3 because of having loose markers (ME > 0.35 mm), and 5 follow-ups because of being covered by osteosynthesis material. The median number of markers was 5 (3–9). The median CN was 46 mm-1 (19–150) and median ME was 0.19 mm (0.04–0.47). 3 of the patients were accepted despite having an ME of > 0.35 mm; all of these patients had an initial ME of less than 0.35 mm as protocolled, but subsequent follow-ups had continuously increasing ME, which was interpreted as minor regional growth.

#### Internal repeatabilities and independency

RPL values from double examinations at 6- or 12-month follow-up (n = 10) with inclusion of screw tips are summarized in Table 2. The median MTPM of these data was 0.34 (0.27–

Table 3. Assessment of RSA stability across the VDRO in relation to RPLs—either if the migration of 2 consecutive follow-ups is below the RPL in all 6 directions or if it is below the RPL in 5 of 6 directions.

Follow-up	Direc	Directions		
(n = 19)	6 of 6	5 of 6		
5-week	15	16		
3-month	16	18		
6-month	18	19		
12-month	18	19		

Table 4. Mean migration and standard deviation at the one-year follow-up of stable VDROs (n = 18)

Direction	Mean	SD
x y z Rx Ry Rz	0.51 mm 0.69 mm -0.21 mm 0.39° 0.02° 2.17°	1.12 1.61 1.28 2.90 3.07 2.29

0.80) mm. Independency between data from bilateral surgeries was tested graphically and interpreted without suspicion of confounding by cluster (Seaman et al. 2014); thus, both sides from patients were included.

#### RSA stability across the VDRO

The results of testing of RSA stability across the VDRO are summarized in Table 3. The table also shows results of what happens if the definition of RSA stability is altered to migration below RPL in only 5 of the 6 orientations

#### Translation and rotation

Table 4 summarizes the mean migration of the RSA-stable VDROs at the 1-year follow-up. Figures 3–8 show the progression of translation and rotation of the femoral shaft distal to the VDRO. In 4 of the figures, there are some outliers (see figure legends for commentary).

#### Maximum total point motion

Figure 9 shows the difference in MTPM between 2 follow-ups ( $\Delta$ MTPM). There are significantly greater migrations at the 5-week follow-up. Decreasing  $\Delta$ MTPM values are observed over the remaining trial period.

#### **Discussion**

Our cohort had similar demography and FHEI to that in other studies (Sankar et al. 2006, Canavese et al. 2010, Davids et al. 2013). The study population therefore seems representative and comparable to that in other studies regarding children with neuromuscular disorders and hip dislocation, but a discrepancy between the perioperatively evaluated varisation and the pre- and postoperative NSA measurements was also found in another study (Geretschläger et al. 2005).

There are practical and technical obstacles to achievement of adequate RSA recordings—especially for the postoperative recording, due to the fixed hip angle in the spica hip cast. For this reason, it was necessary to include screw tips in the femoral shaft as markers, thus increasing the ME values as expected. 3 of the 19 RSA results were accepted, despite having ME > 0.35 mm. This was because they had an initial ME value below 0.35 with small, continuously increasing values above 0.35. We could justify this, since it was interpreted as being due to either remodeling or to minor regional growth. The assumption in including the screw tips as markers seemed plausible, since the MbRSA software would detect loose markers or unstable screw tips from increasing ME. Comparing the RPL to the phantom model results, the RPL approximately doubled

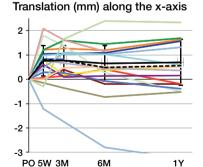
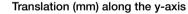


Figure 3. Medial-lateral translation. By our definition of RSA-unstable, the bottom outlier (patient #15) was unstable during the whole trial period with extremely long surgery time because of the need to reposition the osteosynthesis material. By linear regression of the RSA-stable VDROs, we estimated medial progression to be 0.09 mm per year. The dashed line (median), the solid line (mean), and the error bars (SD) do not take account of the outlier. PO: postoperatively; 5W: 5 weeks; 3M: 3 months; 6M: 6 months; 1Y: 1 year.



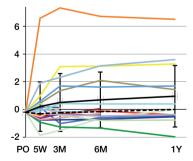


Figure 4. Superior-inferior translation. The top outlier (patient #3) showed large migrations within the first 5 weeks. This VDRO was RSA-stable during the first 3 months. By linear regression of the RSA-stable VDROs, we estimated superior progression to be 0.41 mm per year. The dashed line (median), the solid line (mean), and the error bars (SD) do take account of the outlier.

#### Translation (mm) along the z-axis

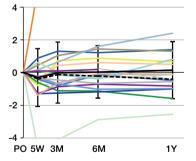
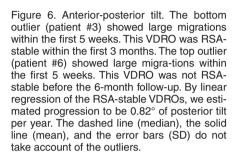


Figure 5. Anterior-posterior translation. The top outlier (patient #3) showed large migrations within the first 5 weeks. This VDRO was RSA-stable during the first 3 months. The bottom outlier (patient #6) showed large migrations within the first 5 weeks. This VDRO was not RSA-stable before the 6-month follow-up. By linear regression of the RSA-stable VDROs, we estimated anterior progression to be 0.10 mm per year. The dashed line (median), the solid line (mean), and the error bars (SD) do not take account of the outliers.

# Rotation (°) around the x-axis



PO 5W 3M

#### Rotation (°) around the y-axis

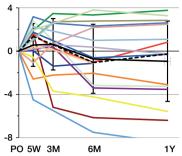


Figure 7. Internal-external rotation. By our definition of RSA-unstable, the bottom outlier (patient #15) was unstable over the whole trial period with extremely long surgery time because of the need to reposition the osteosynthesis material. By linear regression of the RSA-stable VDROs, we estimated progression to be 0.92° of external rotation per year. In contrast to the other migration figures, the mean seemed to bend toward internal rotation after the 6-month follow-up. The dashed line (median), the solid line (mean), and the error bars (SD) do not take account of the outlier.

#### Rotation (°) around the z-axis

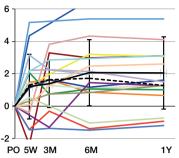


Figure 8. Varus-valgus angulation. There are no outliers singled out. By linear regression of the RSA-stable VDROs, we estimated progression to be 0.71° varus angulation per year. Dashed line: median; solid line: mean; error bars: SD.

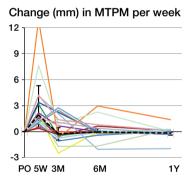


Figure 9.  $\Delta$ MTPM per week across each VDRO. The dashed line is the median MTPM value and the error bars depict the first and third quartiles.

when we included the screw tips as additional markers. We found the RPL of the out-of-plane directions (z and Ry) to be mostly affected by addition of screw tips as markers when using a uniplanar calibration cage, as seen in other studies (Bragdon et al. 2004, Stilling et al. 2012).

The main aim of this study was to assess the stability across VDROs by RSA in children with neuromuscular disorders and syndromes. We defined an osteotomy as being RSA-stable if measurements in 2 consecutive follow-ups had migration below the internal RPL of each of the 6 orientations. We tried to determine whether our definition was adequate by changing it to 2 consecutive follow-ups with migration below the RPL in only 5 out of 6 orientations. However, we found only a marginal difference in RSA stability between these 2 definitions. When using the first definition, 1 of the patients would never achieve an RSA-stable osteotomy throughout the whole

study period. This is contradictory to the clinical history since shortly after ending the study, the patient had the osteosynthesis material removed and continued physiotherapy, and still has no signs of relapse or collapse across the VDRO. This indicates that the second definition of RSA stability may be more appropriate clinically. When considering the statistical aspects, one would have a risk of type-I error in 5% in each orientation. Evaluation of all 6 orientations together under the assumption of being binomially distributed would mean a likelihood of type-I error as high as 26%. There is interdependency between migration in the different orientations, minimizing this risk—but it would still be greater than 5%. A definition of RSA stability of migration above RPL in 2 whole orientations would theoretically introduce a higher risk of type-II error. Thus, the second definition of RSA stability is the better choice. It appears to be the best choice in this context; we can therefore conclude from RSA (and to our knowledge, show for the first time) that the VDROs were stable over the first 5 weeks postoperatively in 95% of those included.

The secondary aim was to estimate the mean migration of the femoral shaft distal to the VDRO in stable osteotomies at the 1-year follow-up. The VDROs showed only marginal total migration, despite the relatively high varus angulation at only 1 year of follow-up. The VDROs initially migrated with a progressive amount of external rotation throughout the first 6 months, but stopped, with slight median internal rotation subsequently from the 6-month follow-up to the 1-year follow-up (Figure 7).

It proved rather difficult to obtain visible markers in the femoral shaft in both RSA radiographs when the patients were still cast-immobilized. We managed to obtain sufficient markers by inclusion of the screw tips in the femoral shaft. Despite this technical difficulty, the RSA results appear to be valid since the internal RPLs are only slightly higher than those in phantom models, and the ME and CN are still within agreed thresholds. The GMFCS classification was used in all the patients included. Originally, the GMFCS classification was only validated for children with CP, but we found it to be applicable and feasible for our patients, since they showed traits of clinical manifestation of neuromuscular disorders with increased risk of hip dislocation (Beckung et al. 2004, Tay et al. 2010). Despite these shortcomings of the study, the results are still important—as they give a better biomechanical understanding of stability, migration, and healing across VDROs. In future studies concerning the definition of RSA stability, our second definition could be tested for applicability on different types of osteotomies and also be used to determine whether the duration of casting could be shortened. The high precision of the RSA method would also be an appropriate tool for assessment of risk factors in long-term relapse.

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PB: study design, data collection, data analysis, writing of the draft paper, and revision of the paper. SSH and NE: study design, surgery, and revision of the paper. CW: study design, surgery, data analysis, and revision of the paper.

No competing interests declared.

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