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Segmental kinematics of manual upper cervical spine axial rotation mobilization:

An in vitro study comparing segmental kinematics of embalmed and unembalmed specimens

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List of abbreviations

ANOVA	Analysis of variance
CC	Cross-correlation coefficient
CCS	Cartesian coordinate system
CS	Cervical spine
e_1	axis fixed to the distal vertebra and coincident with the Z-axis of the proximal vertebra coordinate system.
e_2	axis fixed to the distal vertebra and coincident with the y-axis of the proximal vertebra coordinate system.
e_3	floating axis, perpendicular to e_1 and $e_{3.}$
FHA	Finite helical Axis method
FOB	Flock-of-Birds electromagnetic tracking device (Ascension technologies Corporation USA).
ICC	Intraclass Correlation Coefficients
ISB	International Society of Biomechanics
JCS	Joint Coordinate System
LCS	Local coordinate system
ROM	Range of Motion
ROM Z	Range of motion of coupled flexion-extension movement
ROM Y	Range of motion of the main axial rotation movement
ROM X	Range of motion of the coupled lateral bending movement
rT	retest
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences version 16.0
STC	Standardization and Terminology Committee
T	test
ZEBRIS CMS	Ultrasound-based motion analysis device (Zebris Medizintechnik GmbH Isny, Germany)

O(o)	origin; intersection of the axes Y and y in the reference neutral position.
Y(y)	line passing through the centers of the upper and lower vertebra endplates, and pointing cephalad.
Z(z)	line parallel to a line joining similar landmarks on the bases of the right and left pedicles, and pointing to the right.
X(x)	line perpendicular to the Y- and Z-axis, and pointing anteriorly.
X-axis	(from the anterior centre of the corpus perpendicular to the Z-axis): segmental lateral bending axis.
Y-axis	(perpendicular to X and Z axes): segmental axial rotation axis.
Z-axis	(from right to left transverse process): segmental flexion–extension axis
3D	Three-dimensional
2D	Two-dimensional
n0	rotation vector
$0_2,0_1,0_0$	Finite helical angles
I	observer 1 test vs observer 2 test
II	observer 1 test vs observer 2 retest
III	observer 1 retest vs observer 2 test
IV	observer 1 retest vs observer 2 retest

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Introduction

Neck pain is a common and growing problem which burden significantly to the cost of healthcare. About 67% of people suffer from neck pain at some stage of his life *(Korthals-de Bos et al. 2003)*. Frequently associated with the pain, another typical disturb of neck disorders is reduction of cervical range of motion, *(McNair et al. 2006)* which contributes increasingly to the worsening of the patients bio-psycho-social condition.

In clinical practise, in order to reach best outcomes as possible, an "evidence based" approach of the patient with is problem is deemed pivotal. That is why the necessity to evaluate and to comprehend methods of physical examination and treatment commonly used in this patient population is getting increasingly important *(Dvorak et al. 1992; McCarthy 2001).* However, despite the importance of cervical spine disorders and the interest showed on this field of musculoskeletal therapy there is a lack of knowledge on the kinematics of the cervical spine related to orthopedic manual medicine *(Cattrysse et al. 2007 PhD).*

In manual therapy, regional as well as segmental manual spinal mobilizations are techniques commonly used during therapeutic procedure. These are specific techniques of movement passively induced in a joint with the aims to regain physiological movement of a movement segment, to improve vascular activity and trophic aspects and to normalise the kinematic, static and protective, function of the spine.

Segmental cervical spinal mobilizations are considered useful to limit desired effects to one specific level. Nonetheless, due to the complexity of cervical spine biomechanics and anatomical structure, it is unknown whether it is possible to expect such desired issue and which are the possible risks of such interventions.*(Cattrysse et al. 2007 PhD ch.1)* Therefore, in order to select specific techniques depending on the wanted effect and to avoid undesired consequences, knowledge of segmental three-dimensional kinematics of cervical spinal mobilization is of great importance to clinicians specialized in manual therapy during the administration of a treatment.
However, so far the only studies about these aspects have been accomplished by Cattrysse et al. *(2007 PhD)*. These authors have analysed *in vitro* the kinematic effects at upper cervical spine level of two different segmental mobilization techniques in flexion-extension, lateral bending and axial rotation, comparing the results with the specific kinematics of a regional mobilization technique. To do so, different and innovative measuring methods suitable for the continuous registration of segmental spinal kinematics have been used. *(Cattrysse et al. 2007 PhD ch.1)*
Previously no information has been presented regarding the kinematics of the main and coupled motions during manual passive mobilization of the occipito-atlanto-axial complex. Several studies have investigated the normal movement patterns of this cervical region in cadaveric specimens or in human subjects with 2-dimensional or 3-dimensional experimental analysis. However, each of them showed specific weaknesses due to use of inappropriate or limited methods of registration of the movements *(Panjabi et al.2001, Ishii et al. 2004, Dvorak et al. 1987, Karhu et al. 1999).* Only a few study have studied the kinematic effects of spinal manipulative therapy at the level of the cervical spine, supplying interesting information but unfortunately not sufficient. *(Lee et al.1997)*
At present, a continuous registration and analysis of three dimensional segmental aspects of manual mobilization can be achieved only with an *in vitro* approach. No *in vivo* studies have been performed.

The following study is constituted by two different parts.

- In the first one, three important matters about cervical spine are discussed. The 3-dimensional kinematics and the factors which may induce variability of its normal patterns of coupling movement are described. Moreover, some important methods of assessing the motion of the two complex cervical spine regions are presented.

- In the second part, an experimental study on the specific kinematics of atlanto-axial motion segment during manual regional and segmental mobilization is presented. An *in vitro* analysis conducted by two observers on unembalmed human spinal specimens using ZEBRIS ultrasound–based coordinate measuring system during manual mobilizations techniques in axial rotation is reported. The results are compared with that derived from other previous studies on embalmed specimens with the application of identical mobilization techniques. In this way differences and correlations among the different data are underlined.

1. Literature Study

1.1. Specific topics and research goals

The following literature study has been structured working up the below specified topics which can be considered as the research goals:

1. The first aim is to analyse the 3-dimensional kinematic of the cervical spine on the whole , in order to underline the main features and the principal kinematics differences between the upper and the lower anatomical districts.

2. The second purpose is to evaluate the principal factors that are causes of inter-individual and intra-individual variability in the normal cervical spine kinematics, with specific regard for the occipito-atlanto-axial complex.

3. The third goal is to present some measuring methods that have been developed so far to analyse the kinematic of the cervical spine, showing advantages and disadvantages of each study.

1.2. Search strategy for selection of studies

Study selection was initiated with the aid of the computer-based search engines of *PubMed* and *Web of Science*. Every search was performed without language or time restrictions and each of them concerned the regions of interest of "*cervical spine*", "*upper cervical spine", "atlanto-occipital joint", "atlanto-axial joint".* Within these regions, the principally searched topics were found using the combined search terms of *"kinematics", "biomechanics", "coupled motion"," finite helical axis" "coupling movements" "Range of Motion", "Zebris", "Flock of Bird", "ultrasound", "coordinate system" and "reproducibility"*. The keywords used as "search restrictions" were *"not surgery", "manual mobilization", "manual therapy", "ICC", "in vivo"* and *"in vitro"*.

Retrieved articles were checked for relevance based on the title and abstract. Full text of the selected papers were retrieved in digital version, paper copy or by IBL-request.

1.3. Results

1.3.1. Cervical spine kinematic

The cervical spine represents an anatomical complex which supports, moves and orientates the head in a three-dimensional space. It can be considered as a complex structure in which every joint segment shows a unique morphology and a different coupling of its various components of motion*. (Bogduk 2000)* Understanding of this complex interaction between the kinematics of the two regions of the cervical spine and also knowledge of its related functional anatomy are imperative in clinical practise.

Initially, segmental spinal coupling motions was considered as axial rotations combined with lateral flexions *(Harrison 1998)*. Later, Panjabi et al. *(Oda et al. 1991, White et al. 1990)* defined it as an association of the main rotation or translation motion of a vertebral body about one axis with all the accompanying rotation or translation motions about or along another axis, consistently. According with these authors, biomechanical coupling is 3-dimensional, take place within six degrees of freedom (three translations and three rotations) and can be often described using a Cartesian coordinate system, a right-hand screw system. *(Panjabi et al. 1974)* The 3-dimensional motions in humans were described as correspondent to flexion-extension, rotation and lateral bending forces. The main initial motion can often be accompanied by five additional coupled motions. *(Panjabi et al. 1989)* The helical axis of motion is as alternative to the three rotations and three translations description of intervertebral motion. Using the helical axis of rotation, the motion is described by the position and direction of an axis of motion, together with a scalar translation along this axis and a scalar rotation around it. *(Wu G et al. 2002)*

In 1990 the Standardization and Terminology Committee (STC) of the International Society of Biomechanics (ISB) proposed a standardization in the reporting of joint kinematics data based on the Joint Coordinate System (JCS), first proposed by Grood and Suntay (1983) for the knee joint. *(Wu G et al. 1995).* Standardization of joint motions is very important for the enhancement of the study of motion biomechanics mainly for two important reasons: firstly these JCSs allow to report joint motions in clinically relevant terms, making the application and interpretation of biomechanical findings easier for clinicians. Moreover, the use of these coordinate system allows the comparisons among various studies that usually is difficult because of the lack of a standard for reporting joint motion in the field of biomechanics for human movement.

In order to establish a JCS for a joint Grood and Suntay proposed a precise procedure. Firstly, for each of the two adjacent body segments (the proximal and the distal) a Cartesian coordinate system (CCS) has to be established and its axes are defined based on bony landmarks. Either landmark is identifiable by palpation or with X-rays, follow the ISB general recommendations *(Wu G et al. 1995).* The common origin of both axis systems is the point of reference for the linear translation occurring in the joint, at its initial neutral position. Secondly, the JCS is established based on the two CCSs. Two of the JCS axes are body fixed, and one is ‘‘floating’’. Finally the joint motion is defined based on the JCS.

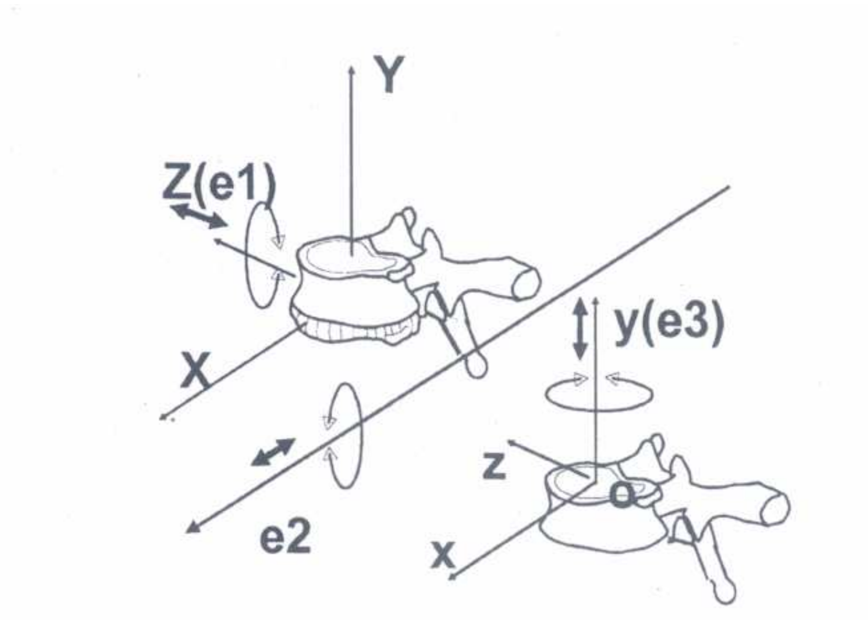

Figure 1. Illustration of a proximal vertebral coordinate system (XYZ), a distal vertebral coordinate system (xyz), and the corresponding JCS.
(Wu G t al. 2002)

- Vertebral coordinate system—XYZ (proximal) and xyz (distal) :

O(**o**): The origin is the intersection of the axes Y and y in the reference neutral position. This neutral position must be in a position where the vertebral axes Y and y are coplanar. If Y and y are parallel the origin O is the mid-point between adjacent endplates.

Y(**y**): The line passing through the centers of the upper and lower vertebra endplates, and pointing cephalad.

Z(**z**): The line parallel to a line joining similar landmarks on the bases of the right and left pedicles, and pointing to the right.

X(**x**): The line perpendicular to the Y- and Z-axis, and pointing anteriorly.

- <u>JCS and motion for the spine</u>**:**

e_1: The axis fixed to the proximal vertebra and coincident with the Z-axis of the proximal vertebra coordinate system.

Rotation (α): flexion or extension.

Displacement (q_1): medio-lateral translation.

e_2: The axis fixed to the distal vertebra and coincident with the y-axis of the distal vertebra coordinate system.

Rotation (y): axial rotation.

Displacement (q_3): proximo-distal translation.

e_3: The floating axis, the common axis perpendicular to e_1 and e_3.

Rotation (β): lateral bending.

Displacement (q_2): antero-posterior translation.

Among many methods proposed to describe 3D-kinematics of human joint movements, a common representation for in vitro spine studies is based on a six degrees of freedom approach usually represented by Euler-Cardan angles (lateral bending, flexion-extension and axial rotation angles). According with some authors, *(Baeyens et al. 2005, Kettler et al. 2004, Cattrysse et al. 2005)* this approach provides a complete description of intra-articular motion, but it produces problems in the therapeutic interpretation of movement due to the presence of some limitations. The three angles are sequence dependent therefore, choosing a different sequence order the motion analysis may result in a completely different description of patterns of coupled motion components. Moreover, the three translation have to refer to fixed a points. Thereby, the Finite Helical Axis (FHA) analysis has been preferred as a valid approach to obtain a functional representation of a joint movement. This axis can be considered as the 3D equivalent of the finite centre of rotation in a 2D analysis of motion and is defined by its orientation, its position, the shift along and rotation about the axis. *(Cattrysse et al. 2007 PhD ch. 3)* However, even though the FHA is useful for the representation of 3D joint kinematics, this approach may cause interpretational problems among clinicians. So that, it has been replaced with the use of the finite helical angles, a mathematical derivate of this approach. The derived angles, are the result of the mathematical decomposition of the rotation around the FHA according to the X-axis, Y-axis and Z-axis of the defined bone embedded coordinate system. The rotation vector n0 (x, y, z) was decomposed in three components corresponding to the helical angles 0_2, 0_1, 0_0. In this way the movement of a motion segment, represented by an angle 0 around a FHA with a direction vector n, could be described as a rotation by these helical angles 0_2, 0_1 and 0_0 simultaneously around three orthogonal axes Z, Y and X, respectively.

The helical angle approach offers some important advantages over other methods. It is not sequence dependent as the Euler-Cardan angle analysis techniques and compared to the FHA representation, it gives an easier interpretation of individual motion coupling patterns and the possibility of comparing group descriptive statistics. *(Cattrysse at al. 2007 PhD ch.3)*

In the following description of the kinematics of the two cervical spine regions, the previously described Joint Coordinate System (JCS) proposed by the International Society of Biomechanics (ISB) will be considered.

1.3.1.1 Upper Cervical Spine

The upper cervical spine comprises the occiput and the first two cervical vertebrae, forming the occipito-atlanto-axial complex. It displays a unique anatomy compared with other regions of the spine and presents the most complicated combination of motions. For this reason, several difficulty have been noticed by several authors during its accurate investigation. *(Ishii 2004-Oda, Panjabi 1991, Assink 2005)*

The atlanto-occipital joint (C0-C1) is a strong union between the head and C1. The atlas functions as an interposed bearing structure between the head and the lower cervical spine, guiding and limiting the movement between the occiput and C2. It is governed essentially by the muscles that act on the head in a passive manner and this is most evident when the main head motion is in sagittal plain rotation (flexion-extension): during this movement usually the atlas exhibits paradoxical motion, i.e. at full flexion of the neck it extends and at full extension it flexes *(Van Mameren et al. 1990).*

According to some authors *(Penning 1978),* this joint functions as an unit allowing essentially only flexion-extension movements (rotation around e_1 axis). At this level axial rotation (rotation around e_3 axis)and lateral bending (rotation around e_2 axis) are not physiological movements because of anatomical limitations. Indeed, in lateral bending the atlas may be rigidly blocked by the shape of its lateral masses, thus forcing this movement of the atlanto-occipital segment in a combination with lateral bending and simultaneous controlateral rotation of the atlanto-axial segment. However , conflicting information about the amount of axial rotation in the atlanto-occipital joint were ensued from several studies. *(Penning 1978, Amiri et al. 2003, Panjabi et al. 1988)*

The union between C1 and C2 forms the atlanto-axial joint. The cardinal function of this junction is to permit a large range of axial rotation and according to several authors *(Penning 1978, Amiri et al. 2003),* this movement mainly take place at this level. The axial rotation requires the anterior arch of the atlas to pivot on the odontoid process and slide around its ipsilateral aspect. *(Bogduk 2000)*

Some authors describe that both the joint surfaces of C1 and C2 are convex and this bi-convexity is generally accepted as an important factor in the determination of atlanto-axial kinematics. However, according to the studies of Cattrysse at al*. (2007 PhD ch. 5)* this behavior could not be demonstrated in vitro during manual mobilization. These authors affirmed that the joint surfaces at this spinal level are nearly flat but with inter-specimen variations from slightly concave to slightly convex. Moreover, no relationship could be demonstrated between the joint surfaces shape and the kinematics of C1-C2. However, the main age of specimens involved in the studies of Cattrysse et al. *(2007)* was 80 years, so the observed flattening of the joint could be related to degeneration induced by age.

During flexion-extension (rotation around e_1 axis) as a main motion, other than the previously mentioned atlas paradoxical movement, antero-posterior translation (q_2) and proximo-distal translation (q_3) coupled motions occur. However, there is no agreement among authors about their direction. Some authors *(Harrison 1998)* reported that flexion is coupled with anterior translation about e_2 axis (q_2) and extension with posterior translation about e_2 axis. Instead, the coupled proximo-distal translation (q_3) direction may not be established because it depends on the origin of motion chosen relative to the vertebral body. On the other hand, from the studies from Oda et al. *(1991)* on C0-C1 translation movements the same problem emerges determining antero-posterior translation (q_2) direction, that is why it may not be preciously determined.

When the main motion is lateral bending (rotation around e_2), several authors *(Mimura et al. 1989, Oda et al. 1991, Penning 1978, Harrison 1998)* have found in C0-C2 a coupled controlateral axial rotation (rotation around e_3): the head and C1 rotate to the opposite side with respect to C2, facilitating the simultaneous main motion. Moreover, Panjabi et al. *(Oda et al. 1991)* showed that during lateral bending, the occiput (C0) extends on the atlas and translates anteriorly about e_2 axis (q_2) and laterally about e_1 axis (q_1) in opposition to the lateral bending side. In the same time C1 flexes (forward rotation around e_1) relative to C2.

According to several studies *(Mimura et al. 1989, Oda et al. 1991, Penning 1978, Harrison 1998, Ischii et al. 2004),* during the main axial rotation (rotation around e_3) opposite lateral bending (rotation around e_2) occurs at C0-C2. Only Iai et al. *(1993)* affirmed that this is truth only for C1-C2 because C0-C1 moves in the same direction of the main axial rotation. Moreover, with the main axial rotation coupled extension (backward rotation around e_1) at C0-C3 were found and also a coupled anterior translation about e_2 axis (q_2) at all levels. The study from Oda et al. *(1991)* on translational motions, demonstrated that C0 translates anteriorly (q_2) and laterally (q_1) in the same axial rotation side and C1 shows a more complicated motion due to biconvexity of the articulations between C1 and C2. This mechanism includes two phases: in the initial stage of the head rotations around the e_3 axis, the atlas translates superiorly; as the magnitude of the main rotation increases, the atlas translates inferiorly on the dens. In contrast, the atlas translations about the e_1 axis (q_1) relative to C2 are not possible because of the presence of capsules of the lateral atlanto-axial joints and mainly of the alar and transverse ligaments. Actually it is not yet completely clear whether these ligaments do attach to the atlas or nut but they can limit secondarily its range of motion by limiting primarily the movement of the head *(Cattrysse et al. 2007, Crisco et al. 1991, Harrison 1998).*

1.3.1.2 Lower Cervical Spine

The lower cervical spine (C2-T1) is considered as the typical cervical spine, where all vertebrae present the same morphological and kinematic features. This region functions as a unit because the muscles are coherent or interwoven and each of them activates several segments. Mobility is maximal in the C5-C6 segment and minimal in the C2-C3 segment (Penning 1978). Each motion segment consists of two adjacent vertebrae and the disc in between. This results in three joints per segment: the intervertebral joint between the vertebral bodies and the disc and two facet joints between the articular processes. This makes the spine both stable and flexible. The cervical intervertebral joints are saddle joints: they consist of two concavities facing one another and set at right angles to one another *(Penning 1978).*

In the typical segments of the lower cervical spine the movement is guided by the biomechanical characteristics of the intervertebral joints: the oblique orientation of the zygapophysial facet joints and the presence of the uncinate processes. The movement of an articular facet can be described as a combination motion components: in flexion it is accompanied by an upward (about e_1 axis) and forward (about e_2 axis) translation and in extension a downward (about e_1 axis) and backward (about e_2 axis) translation. Lateral bending (rotation around e_2 axis) is a combination of upward movement on one side and downward movement on the other, and automatically this involves the axial rotation (around e_3 axis) that can be considered as a forward movement on one side and a backward movement on the other side. *(Penning 1978)* So, when the main motion is lateral bending, according to several authors *(Mimura et al. 1989, Oda et al. 1991, Penning 1978, Harrison 1998)*, in C2-T1 a coupled axial rotation occurs to the same side. Indeed, when a person attempts to make an axial rotation in the horizontal plane, this movement is coupled with ipsilateral side flexion *(Harrison 1998, Mimura et al. 1989, Bogduk 2000, Ishii et al. 2004, Penning, 1978, Panjabi et al. 2001)* and the head is kept upright by the compensatory lateral flexion of the upper cervical spine in the opposite direction *(Penning, 1978).* Additionally, during the main axial rotation at C3-C7 a coupled flexion, anterior translation about e_2 axis (q_2) and coupled lateral translation about e_1 axis (q_1) occurs in the same direction of the main motion due to the ipsilateral side flexion of the skull at these levels *(Mimura at al. 1989).*

1.3.2. Variability of the cervical spine kinematic

Several authors *(Panjabi et al.1993, Feipel et al.1999)* considered the coupled motions as valid indicators of changes in the normal spinal kinematics allowing the quantification of musculoskeletal impairments. Variations in quantity and in quality of coupling behavior reportedly identify potential risk factors and depict important clues to locate spine pathology *(Cook et al. 2006)*. On the other hand, other authors have affirmed that coupling motion is not clinically useful during diagnosis because the motions involved during coupling are very small and it can be excessively difficult to determine for the manual clinicians. *(Ishii et al. 2006).*

In order to better interpret observations and to select suitable treatment modalities, it is necessary to know not only the normal movement patterns but also the main factors which may involve variations intra-individual or inter-individual of the normal coupled motion. *(Dvorak et al. 1992; McCarthy et al. 2001)*

According to results of some studies *(Panjabi et al. 1993, Walmsley et al. 1996 Panjabi et al. 1989, Harrison 1998),* behaviours of 3-dimensional motion of the upper cervical spine vary with spinal region and vertebral level, and moreover they may be altered by combined postural loading. Walmsley et al. *(1996)* analysed the effects of altering the sagittal plane posture on the available axial rotation, demonstrating that its amount was reduced in all postures compared with the neutral position. This indicate that the static sagittal plane alignment of an individual directly influences the range of motion of the intervertebral joint as a result of a change in vertebral position, increased loading and change in strains of the tissue governing the movement. Also Panjabi et al. *(1993)* affirmed that changes in the position of the skull in the sagittal plane can bring about alterations of coupling amount and direction at occipito-atlanto-axial complex level and it is affected differently depending upon which main rotation was applied. Some motions remained unchanged, while others either increased or decreased. For instance, according to authors results, concerning C1-C2 the main motion of lateral bending decreased significantly from about 10° at to about 0° in flexed posture. Also the coupled axial rotation underwent a decrease from full extended and neutral posture to full flexion moreover changing the direction.

Also Edmondston et al. *(2005)* affirmed that there is a variability in the commonly described stereotypical pattern of ipsilateral movement coupling between cervical spine axial rotation and lateral flexion and the posture in which movements are initiated appears to have a significant influence on this. These authors observed variations particularly when movements were performed in the protracted and retracted postures.

Moreover, again other authors *(Bogduk 2000)* affirmed that the segmental range of motion is not stable with the time and it differs according to the initial motion chosen.

All these observations may have important implications during assessment procedure. For example, in the case of patients with kyphotic cervical configurations the application of lateral bending and axial rotation as main motions can result in coupling patterns that are different from those of people with a normal lordosis. However, the differences would not be a segmental problem or altered motion but rather it would be normal coupling for abnormal static sagittal plane configuration of the cervical spine. *(Harrison 1998)*

Other factors which may influence cervical spine motion characteristics and mechanical responses are joint degeneration and the age of the patient *(Trott et al. 1996, Van Roy et al. 2004)*. A decrease of the cervical ROM and mainly of the main motion could be induced by the latter factor.

The effect of sex on cervical ROM is still largely discussed. Some studies report no significant sex-related variations *(Cagne et al. 2007, Chen et al. 1999)* other *(Castro et al. 2000)* found only significant differences between women and men in older subjects between the ages of 70 and 79 years in flexion and extension and in lateral bending. It was also found that women in advanced age were significantly more mobile than the respective group of men of the same age.

Some anatomical studies *(Van Roy et al. 1997, Van Roy et al. 2001)* demonstrated that various features of cervical vertebrae and inter-vertebral joints reveal large inter-individual morphologic differences. Therefore other authors *(Cattrysse et al. 2007 PhD ch. 2,3,4; Bogduk 2000)*, relating the cervical spine kinematic to the structure of the cervical vertebrae joint surfaces and ligaments, have explained the inter-specimen variation observed in coupling patterns with this inter-individual variability in specific anatomical features.

Later however, with another study Cattrysse at al. *(2007 PhD ch. 6)* have obtained results which have not confirmed completely that was affirmed in the previous one. Indeed, in the latter study it has been demonstrated that the anatomical features (as the left-right asymmetries in the orientation of joint surface of C1-C2 and asymmetry of the alar ligaments with reference to the sagittal reference plane of the axis) can influence only partially (about 50%) the characteristics of the range of motion of the main axial rotation and the coupled motion components. As a consequence of that, it could be affirmed that variation in the patterns of motion coupling between main and coupled movement components could be also due to intervention of the therapist itself during manual mobilizations.

Thus, during testing segmental motion and treating limitations, a clinician should take care of the postural position of the cervical spine and many other factors; one must interpret carefully a single alteration of range, because it could be related to a disease or to the effect of a therapeutic intervention but it could be as well related to normal anatomical variation. *(Bogduk 2000)*

1.3.3 Measuring methods

So far, several studies using *in vitro* and *in vivo* set-up have been performed with the purpose to analyse the cervical spine mobility and many attempts have been made to obtain an objective method of assessment. In order to reach this goal a large amount of non invasive methods for measuring ROM with varying degrees of accuracy and repeatability have been commercially available. However, there is little agreement among researchers and clinicians about which method should be chose to obtain a better cervical ROM evaluation. *(Cagne et al. 2007)*

Electro-goniometers and electro-inclinometers *(Tousignant et al. 2001, Edmondston et al. 2005)*, Cervical Range of Motion device (CROM) *(Capuano-Pucci et al. 1991; Youdas et al. 1991)* tape measures, visual estimation, ultrasonography-based systems, optoelectronic systems and computer interfaced video imaging are widely used for clinical purposes. However, several studies concerning the reliability and validity cervical spine mobile have demonstrated that most of these methods are seriously flawed. *(Bergman et al 2005, Strimpakos et al 2005)* Most of these instruments, although cheap and easily applicable in a clinical setting, are limited as they cannot build composite pictures of combined planes of motion that also take into account velocity of movement *(Jordan et al. 2000).* Also, many of them are subject to bias from extraneous motion introduced from the thoracic spine and the palpation of anatomical landmarks for instrument application introduces an inherent source of experimenter bias *(Tousignant et al. 2001).* Other instruments utilizing electromagnetic and optoelectronic technology, despite the fact that they have allowed a complete kinematic investigation of cervical spine, there are not useful in every day clinical practice due to their complicated measurement procedures and analyses and their cost too high for clinicians. *(Strimpakos et al 2005).*

A significant development in measuring cervical motion has taken place with the introduction of 3-dimensional motion analysis systems that can record, calculate, and display spatial head position. These systems can monitor relative changes in curvature of the spine during movement as well as to limit researcher bias because the results are displayed graphically and in tabulated forms on screen. *(Strimpakos et al 2005, Wang 2005)* One of these systems is the ZEBRIS, an ultrasound–based coordinate measuring system developed in Germany that appears to be one of the best devices available at the moment to measure cervical ROM in three dimensions. *(Cagne et al. 2007,Castro et al. 2000; Dvir et al. 2000)*

Figure 2. ZEBRIS CMS system (Zebris Medizintechnik GmbH Isny, Germany)

Different types of ZEBRIS CMS system (Zebris Medizintechnik GmbH Isny, Germany) have been developed: CMS 70, CMS 50 and CMS 20.
The most recent version among the above mentioned is the family of CMS 20 measuring system so, it will be described.

This system of motion analysis is designed to measure the travel time of the ultrasound pulses. The subjects under exam sat on a chair with feet on the floor and the trunk fastened to the back of the chair. The subject wears a helmet and a shoulder cap on the right shoulder that serve as reference plane. Each one are fitted with three ultrasound microphones. These ultrasound microphone markers receive signals from the transmitters located in the measuring unit. The measuring unit is positioned on a stand approximately one meter to the right of the subject and sampled by a computer at 20 Hz. The ultrasound transmitters send continuous pulses. The ZEBRIS analyzer analyzes position according to the principle of the timing of the interval between the emission and the reception of ultrasound pulses. By triangulation, the absolute three-dimensional coordinates are calculated. *(Malmström et. al 2003, Wang et al. 2005, Strimpakos et al. 2005)*

There are many advantages of using an ultrasound based motion system to record the motion. *(Wang et al. 2005)* This system employs built-in markers on the shoulder or head attachment instead of using individual reflective markers, and no individual bony landmarks have to be verified reducing the preparation time. In addition, unlike other instruments to assess human movements (including Vicon system, X-ray et al) which need high cost and hard-moving, Zebris system is a convenient and simple to handle instrument. The CMS equipment used in a clinical setting is more cost-effective than other sophisticated motion analysis systems such as the Vicon system. Hence, it is ideal for application in routine operation in human motion measurement. *(Malmström et al. 2003)* Moreover, Zebris device is reliable and valid, as verified by several authors.

Mannion et al. *(2000),* analysing the cervical spine ROM of nineteen volunteers subjects, studied the day-to-day reliability of this computerised motion analysis device. They found a good test-retest reliability for this instrument: there was no significant difference between the mean values derived on the two separate days ($P>0.05$), and the corresponding intra-class correlation coefficients (ICC) ranged between 0.75–0.93 for all primary movements (flexion-extension, lateral bending and axial rotation in neutral position) and between 0.57–0.93 for axial rotation in flexion and in extension.

MalmstrÖm et al. *(2003)* performed active cervical kinematics analysis with the ZEBRIS CMS 30/70P device in sixty asymptomatic volunteers. They accomplished the test-retest in the same occasion, so with more similar musculoskeletal conditions than if the test and the retest had been performed on different days. However they obtained high ICC values between the test and the retest in accordance with those of Mannion et al. *(2000)*: the single-measure ICC values ranged from 0.93 to 0.96 for full-cycle measurements and between 0.76 and 0.96 for half-cycle measurements, with the lowest ICC for right rotation and the highest for extension. In the present study the authors compared this non-invasive ultrasound motion device (ZEBRIS) with the Myrin gravity-reference goniometer, obtaining results that showed reliability and agreement between each other. A single-measure ICC for full cycles ranged from 0.93 (lateral flexion) to 0.96 (flexion–extension) and for half cycles from 0.78 (right rotation) to 0.92 (extension).

Also in Cagne et al. study *(2007)*, the ultrasound-based motion analysis system was shown to be a reliable tool for continuous tracking of cervical spine primary motions. In order to test the intra- and inter-rater reliability, 12 out of 126 volunteers (96 healthy subjects, 14 patients with idiopathic neck pain, and 16 patients with chronic whiplash) were analysed with the ZEBRIS CMS 70P. Results demonstrate a high degree of test-retest reliability in measuring cervical ROM: the single-measure ICC ranged from 0.80 to 0.94 for full-cycle measurements and between 0.50 and 0.92 for half-cycle measurements.

Wang et al *(2005)* measured twice cervical ranges of twenty Chinese adults in order to test the intra-session test–retest reliability of ZEBRIS system CMS 70P in the six directions of the three cardinal planes. The analysis was done by the same rater in the same day at 10 min intervals. The ICC values of the intra-session test–retest reliability of the six principal cervical motions ranged between 0.85 and 0.95. Instead, to measures the inter-session reliability other twenty-eight healthy adults were repeatedly measured by the same rater within a 2 week interval. ICC values of the intersession reliability from 0.58 to 0.88 were found.

Strimpakos et al. *(2005)* by using a ZEBRIS CMS20 ultrasound-based motion analysis device, assessed thirty-five healthy subjects in all neck movements from sitting and standing initial positions, actively and passively. Three tests were employed to assess intra-examiner reliability and two examiners used for the inter-examiner reliability. X-rays in neck flexion and extension were used to validate the ZEBRIS system. The standing position gave higher ICC values (>0.86) than sitting (>0.79). Passive assessment of neck ROM presented better reproducibility than active assessment in both positions. The inter-examiner reliability presented moderate ICC values ranged from 0.43 to 0.68 probably due to the experience level of the investigators.

However, despite the ZEBRIS system is a very capable tool and convenient in its application to subjects, it also presents some limitations. It is not able to measure segmental cervical motion and it can only record spinal and upper extremity motions confined to a relatively small space (within 3x3x3m). *(Wang et al. 2005)* Moreover there is a lack of randomization due to the software routine and in very mobile subjects the contact between the transmitters and the stable microphones can be lost (especially in axial rotation and lateral flexion). *(Strimpakos et al. 2005)* Furthermore, some female participants in some studies experienced a sensation of dizziness, annoyance or other similar inconveniences during the tests. *(Strimpakos et al. 2005, Dvir et al. 2000)* The cause of these symptoms is not clear.

Another relatively new technique for measuring the cervical ROM is the "Flock-of-Birds" (FOB), a six-degrees-of-freedom electromagnetic tracking device (Ascension technologies Corporation USA).

Figure 3. "Flock of Birds" electromagnetic tracking device

This system consists of one standard range transmitter and three receivers mounted one on a stylus and the two other on the forehead and sternum respectively . The first receiver is used for palpation of seven bony landmarks on the head and thorax. *(Meskers et al. 1998, Meskers et al. 1999)* With the positions of these landmarks, one local coordinate system (LCS) of the head and one local coordinate system (LCS) of the thorax are constructed, defining the posture of the patient. This definition of landmarks makes the measurements less dependent on exact positioning of the two receivers on the head and thorax, and makes follow-up measurements more accurate. The other two receivers measure the change of position and orientation in the electromagnetic field while moving. Mathematically, mobility is defined as movement of the coordinate system of the head relative to the coordinate system of the thorax. Before the utilising, a position-calibration procedure has to be performed because the disturbance produced by metals in the environment, such as iron-strengthened concrete, can influence the measurements *(Meskers et al. 1998, Meskers et al. 1999).*

Recently, in the Centre for Rehabilitation of the University Medical Centre Groningen Netherlands, Koerhuis et al. *(2003)* analysed the accuracy and the reliability of the FOB system for measuring cervical ROM. In ten normal subjects axial rotation (in neutral, in maximally extended and in maximally flexed position), flexion-extension, and lateral flexion were analysed actively and passively. To assess the reliability each movement was repeated eight times. To test the accuracy of the measurement system a "dummy head" was used. These measurements indicated that the FOB is an accurate measurement system for neck movement with a maximal error of 2.5° over a range of 180°. The reproducibility of axial rotation, forward flexion, and lateral bending was within 0.85° and was within 1.7° for combined movements such as axial rotation in flexed or extended position. However, a small variation (2°-4°) in the same session and a substantially larger variation between two measurement sessions (5°-16°) were found. Moreover, this study included a small size of asymptomatic samples and it was not established the inter-observer reliability .

More recently, Assink et al. *(2005)* defined the inter-observer reliability of the Flock-of-Birds system testing symptomatic and asymptomatic human subjects, actively and passively, in rotation (in neutral, flexed position and extended positions), flexion-extension and lateral bending. They found the reliability as sufficient for measuring cervical spine active rotation in the neutral position, flexion-extension, and lateral bending: the ICC values ranged from 0.57 to 0.85 for asymptomatic subjects and from 0.36 to 0.91 for symptomatic subjects.

The FOB system has proved to be a practical system. The sensors are small, 2- 2,5-2,5 cm, and in spite of the cable connections the encumbrance of the subjects is minimal. However, disadvantages of electromagnetic sensors are that the measurement space is confined to a short distance from the (standard range) transmitter, that it should be free of magnetic materials and that an extensive calibration is necessary. So this implies that the system is not portable in practice. *(Koerhuis et al. 2003)*

The “Flock-of-Birds” system also was utilized by Cattrysse et al. *(2007)* in order to investigate in vitro the kinematic effects of two different segmental mobilization techniques in axial rotation, side bending and flexion-extension mobilizations in the upper cervical spine. The authors used this electromagnetic tracking device in combination with the Microscribe G2X (Immersion Corporation, USA), a 3D digitising stylus device, with an accuracy of 0.009” (0,23mm) and a workspace size of 50”(1,27m), that offer the possibility to register 3D features of an object in order to reconstruct the object digitally or process the data mathematically. Thus the kinematic results were related to a bone embedded reference frames defined on the upper cervical spine segments.

1.4. Conclusion

In this first part only few but important facets regarding the cervical spine on the whole have been portrayed. The complexity of the kinematics of the upper and lower cervical regions and the general problem in precisely describing their variable interactions are extremely evident in all reported studies, underlining the essential necessity of a continuous underdeveloped research on this field of the Manual Therapy.

2. Experimental Study

2.1. Introduction

A careful analysis of the kinematics of the upper cervical spine during manual passive mobilization techniques has a large important in clinical practice.

However, despite the importance that has been addressed to these aspects, only recently they have been analysed *(Cattrysse et al. 2007).*

So far, a large number of studies in an *in vitro* and *in vivo* set-up have been able to investigate just the normal cervical moving patterns.

In the present study an *in vitro* analysis on twenty fresh human spinal specimens in a test–retest situation with two observers is presented. The results derived from the combination of using of an ultrasound device for continuous motion registration with manual mobilizations techniques are reported and compared with which arise from other previous *in vitro* studies on embalmed specimens *(Cattrysse et al. 2007 PhD ch. 1 part 1 and ch. 4).*

Aim of this examination is to analyse:

- the reproducibility of three different manual axial rotation mobilization techniques and the differences and correlations among them.
- the differences and correlations between segmental kinematics of embalmed and unembalmed specimens during application of manual axial rotation mobilization techniques.

All the analysis have been performed at C1-C2 level (atlanto-axial joint).

As this experimental part of the study includes the analysis of results from the studies of Cattrysse et al. *(2007)* the methodological part is identical. The following information is derived from Cattrysse et al. *(2007 PhD ch.1, ch.3 and ch.4)* studies.

2.2. Methods and materials

2.2.1 Specimens:

Twenty fresh human spinal specimens were included in this study. Nine specimens from male and eleven from female subjects. Each specimen consisted of the occiput, all the cervical segments and the first two thoracic vertebrae. Specimens were a mean of 80 years (+/-11 years) with a range 59–97.

Room temperature was controlled between 15° and 20°C and humidity was above 60% to avoid dehydration of the specimens during testing. The specimens were rapped in saline moistened towels during the registration set-up and between tests. The wraps were removed before mobilization to provide full free movement of the segments.

2.2.2 Instruments:

An adapted ZEBRIS CMS 20 ultrasound-based motion tracking system (Zebris Medical GmbH – Germany) was used in this study. The accuracy of the system has been studied using a single hinge phantom. One transmitter and the receiver of the device were mounted on a high accuracy rotary stage (Time and Precision Ltd., Baringstoke, England) making it possible to produce angular displacements with an accuracy of 0.02° per step. A hinge joint motion around a fixed axis differs from complex daily life motion of joints. A hinge joint phantom was preferred to analyze the accuracy of the tracking

system on the axis of motion and the cross-talk and error effects on the coupled motion axes.
The standard deviations can be used as an indicative measure of error. An overall deviation of 0.04° occurs on the main axis on a total measurement range of 75° of motion of the phantom. Standard deviations of 0.25° and 0.29° occur on the other axes. Differences between the performed angular displacements and the angles calculated can be partly attributed to cross-talk effects. After applying a correction technique for misalignment between the axis of the phantom and the reference frame defined during the set-up of the Zebris system, based on an optimization technique *(Cattrysse et al. 2007)* these standard deviations for the real and the measured angles can be reduced to 0.20° and 0.13°. The system thus reproduces angles of movements with an accuracy of less than 0.1° for the main motion component and 0.2° for the coupled components.

2.2.3. Methods:

In order to prevent limitation of movements and uncontrolled coupled motions that the fixation of the ultrasound system on the segments might induces, all the skin, subcutaneous tissue and muscles were dissected, keeping the muscular insertions and ligaments intact.
It has been demonstrated that the biomechanical properties of the tendons and ligaments do not change due to conservation by freezing *(Panjabi et al. 1985, Wilke et al. 1996)*, therefore, the possibility of bias on the results due to biomechanical changes within the muscles was reduced as much as possible.
Specially fabricated fixation tools were inserted in the parietal part of the occiput, the transverse process of the atlas and the transverse process of the axis. The transmitters and receiver of the ZEBRIS system were mounted on

these fixation tools. The optimal positioning of the device was controlled for every specimen prior to the start of the mobilizations. Fixation pins were drilled cross linked through the corpus of T2. The specimen was mounted in a wooden frame by these fixation pins. In this way the specimen was positioned as if the subject was in a supine position on an examination table. The preliminary dissection and the optimal positioning of the fixation tools assured free mobility of the cervical spine trough full range of motion in axial rotation, lateral bending, flexion–extension and combined directions.

Figure 4. Experimental set-up with the specimen in supine position and fixation of the ZEBRIS ultrasound system.

Firstly each specimen was mobilized in a planar way trough the full range of cervical axial rotation mobility.
Subsequently two different segmental mobilization techniques were performed at the level of the atlanto-axial joint (C1-C2):

- the first one, labelled as the "fixation" technique [Figure 5(a)] in this study, implies the manual axis fixation during manually induced rotation to the left and to the right of the atlas;

- in the second technique, labelled as "locking" [Figure 5(b)] in this study, the cervical spine was brought in a 3D locking position leading to a bony or capsule-ligamentous locking of the joint. Combining lateral bending and axial rotation of all inferior cervical segments till the level of the axis, the motion in the adjacent segments is minimized. In this position the atlas was mobilized in axial rotation with respect to the axis.

All the mobilization techniques were performed three times consecutively by two investigators with several years of experience in manual therapy, in a test–retest situation. One of the examiners was familiar with the examined techniques for many years. The other usually performed similar but not identical mobilizing techniques and familiarized with the specific techniques described above before the testing period.
The test–retest order was assigned randomly for the two investigators. Investigators were blinded from the analysis data of the system during testing.
Both examiners performed a trial with feedback of the tracking system in a test–retest situation on one specimen to familiarize with the techniques and the test set-up.

Figure 5. **Segmental manual mobilization of the atlanto-axial joint:**
(a) with manual fixation of the axis
(b) with combined locking of the lower cervical spine.

2.2.4. 3D angles of motion:

The angle of movement used in this study are the angles reproduced from the Zebris-winbiomechanics software. A graphical representation of the calculated angels has been presented by Wang et al. *(2005).* The definition of the local reference frame used by the ZEBRIS system is based on three markers L, R and F. The point L (left) was chosen on a marker inserted on the left transverse process of the axis, the point R (right) on the right transverse process and the point F (front) centrally on the anterior side of the corpus.
Although the International Society of Biomechanics (ISB) provides guidelines defining the local reference frame for mid cervical spinal segments it does not define standards for local reference frames on the atlas, or axis *(Wu et al., 2002)*. Due to the nature of the experiment and the specific anatomy of the upper cervical vertebrae the centre of the corpus could not be defined. The above described frames for atlas and axis were therefore defined and the labelling of the axes was chosen in congruency with the ISB guidelines.

The axes are defined as follows:

- Z-axis (from right to left transverse process):
  segmental flexion– extension axis.
- X-axis (from the anterior centre of the corpus perpendicular to the Z-axis): segmental lateral bending axis.
- Y-axis (perpendicular to the X and Z axes):
  segmental axial rotation axis.

The direction of the Z-axis was reversed to create a right handed orthogonal reference frame. For reasons of clearness of the graphical and numerical representation the sign of the angles around the Y-axis was changed. In this way an axial rotation and a lateral bending to the same side are indicated by the same sign (left and right, respectively, represented by - and +signs)

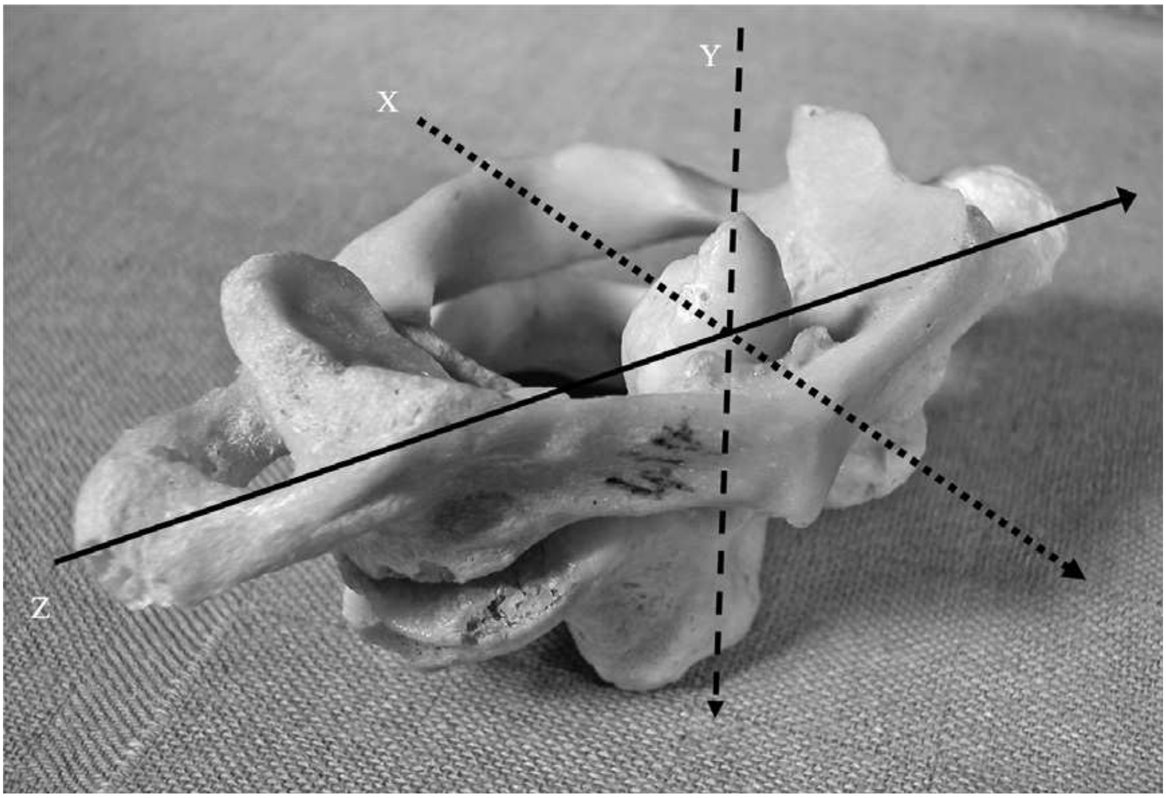

Figure 6. **Bone embedded coordinate system on C1:**
X-axis (segmental lateral bending);
Y-axis (segmental axial rotation);
Z-axis (segmental flexion–extension).

2.2.5. Data analysis of motion coupling patterns:

The patterns of motion coupling between the main axial rotation motion and the coupled lateral bending movement component were analyzed. Six different parameters were defined to describe these coupling patterns in an objective way:

- ROM Z: range of motion of coupled flexion-extension movement
- ROM Y: range of motion of the main axial rotation movement
- ROM X: range of motion of the coupled lateral bending movement
- CC: cross-correlation coefficient between the main axial rotation and the coupled lateral bending. This parameter reflects the congruency between these two component of movement and ranges from -1 to +1. It can be regarded as the equivalent of a Pearson correlation coefficient.
- Ratio: ratio between the standard deviations (SD) of the main axial rotation and coupled lateral bending motion components; thus depicts the ratio over the whole course of the mobilization.
- Shift: the unintended delay of the start of the coupled motion with respect to the main axial rotation motion as a fraction of the total period of the main movement (expressed in percentages). It can be demonstrated that this shift equals the arc cosine of the modulus of the cross-correlation coefficient.

2.2.6. Statistical analysis:

For all statistical calculations SPSS 16.0 software was used.

An ANOVA was performed for three different mobilizing techniques separately analysing the six parameters, in order to underline the presence of differences between the two examiners data in the test and retest situation *(Table 1)*. A Kolmogrow-Smirnoff goodness of fit test was performed to control the normal distribution of data within these six parameters and descriptive statistics were calculated *(Table 2)*. The reproducibility of the results was studied by analyzing differences as well as correlations between test and retest results. The presence of differences between the mean of the three mobilization techniques was analysed with ANOVA and for all parameters that presented differences a Student's t-test for Paired-Samples was performed, with Bonferoni adjustment ($p=0.025$). The strength of the correlation between parameters in different measurements situations was estimated by the Intraclass Correlation Coefficients (ICC). It is calculated as the ratio of the variance within subjects (subject variability) over the variance within subjects and variance between subjects. ICC values can be interpreted according to the following classification: <0 is 'poor', 0–0.20 'slight', 0.21–0.40 'fair', 0.41–0.60 'moderate', 0.61–0.80 'substantial' and 0.81–1.00 'almost perfect'.

Significance was tested using the 5% rejection level ($p < 0.05$).

2.3. Results

The results of the Kolmogrow-Smirnoff goodness of fit test performed to analyzed the six parameters showed no significant departures from the normal distribution. Therefore parametric statistical techniques were used. Results of the ANOVA performed to compare the data of the two examiners in the test and retest situation, showed that almost all parameters presents highly significant differences between situations ($p<0.01$) in all three different mobilization techniques separately; only for the cross-correlation parameter (CC) the values were not significant. *(Table 1)*

Table 1: ANOVA results reproducibility study for three atlanto-axial axial rotation mobilizing techniques.

Techniques	Regional	Fixation	Locking
Parameters	*sign*	*sign*	*sign*
ROM X	**0.000**	**0.000**	**0.015**
ROM Y	**0.003**	**0.018**	**0.040**
ROM Z	**0.000**	**0.000**	**0.002**
CC	**0.341**	**0.977**	**0.536**
Ratio	**0.002**	**0.007**	**0.000**
Shift	**0.038**	**0.005**	**0.001**

ROM Z: range of flexion-extension motion component; *ROM Y*: range of main axial rotation component; *ROM X*: range of lateral bending motion component; *CC*: cross correlation coefficient; *Ratio*: ratio between axial rotation and lateral bending motion component; *Shift*: shift between coupled lateral bending component and main axial rotation motion component

Significant values ($p<0.05$) are depicted in light azure field

Highly significant values ($p<0.01$) are depicted in dark azure field

The ICC’s for intra-examiner results and inter-examiner comparison of data are summarized in *Tables 2(A-B)* and *Table 3* and below described separately for each of the three atlanto-axial axial rotation mobilizing techniques.

Regional mobilization:

- The intra-examiner comparison for the observer 1 shows *(Table 2 A)*:

significant relationship for all the parameters between the test and retest results, except for the range of motion of the coupled flexion-extension motion (ROM Z). However, the ICC’s values are “moderate” for CC (Cross Correlation), Ratio and Shift while for the range of motion of the coupled lateral bending (ROM X) and of the main axial rotation motion (ROM Y) are “substantial”.

- The intra-examiner comparison for the observer 2 shows *(Table 2 B)*:

significant relationship only for ROM Y and Ratio parameters between the test and retest results, with “substantial” ICCs values.

- The inter-examiner comparison indicates *(Table 3)*:

“substantial” correlation (mean ICC 0.71) between the results of the two observers for the ROM Y parameter; “almost perfect” correlation (ICC 0.80)for the ROM Z parameter only in the I situation (observer 1 test vs observer 2 test); “substantial” relationship (ICC 0.64) for the CC only in the II situation (observer 1 test vs observer 2 retest).

Fixation technique:

- The intra-examiner comparison for the observer 1 displays *(Table 2 A)*:

significant relationship only for ROM Y and ROM Z and Shift parameters between the test and retest results, with "substantial" ICCs values for ROM Z and Shift and "almost perfect" for ROM Y.

- The intra-examiner comparison for the observer 2 shows *(Table 2 B)*:

significant relationship between the test and retest results only for ROM Y, with a "substantial" ICC value (0.62)

- The inter-examiner comparison indicates *(Table 3)*:

"substantial" correlation (mean ICC 0.71) between the results of the two observers for the ROM Y parameter; "moderate" correlation (ICC 0.54) for the ROM Z parameter only in the I situation (observer 1 test vs observer 2 test); "moderate" correlation (ICC 0.57) for the ROM X parameter only in the IV situation (observer 1 retest vs observer 2 retest); "substantial" relationship (ICC 0.70) for the CC only in the II situation (observer 1 test vs observer 2 retest).

Locking technique:

- The intra-examiner comparison for the observer 1 displays *(Table 2 A)*:

significant relationship for all the parameters between the test and retest results, except for ROM Z and Ratio parameters; the results show "moderate" correlation for CC (ICC 0.55), "substantial" relationship for ROM Y (ICC 0.66) and for ROM X (ICC 0.79) and “almost perfect” (ICC 0.80)correlation for shift parameters.

- The intra-examiner comparison for the observer 2 shows *(Table 2 B)*:

significant relationship between the test and retest results only for ROM Y and Ratio parameters; there is “substantial” reproducibility for ROM Y (ICC 0.67) parameter and “almost perfect” (ICC 0.82) correlation for Ratio parameter.

- The inter-examiner comparison indicates *(Table 3)*:

“substantial” correlation (mean ICC 0.64) between the results of the two observers for the ROM Y parameter; only in III situation (observer 1 retest vs observer 2 test) there is no significant relationship. “Almost perfect” correlation (ICC 0.83) for the ROM Z parameter only in the I situation (observer 1 test vs observer 2 test).

The range of the main axial rotation motion (ROM Y) is the only parameter that shows significant results in inter-examiner (mean ICC 0.69) and in intra-examiner comparison of both observers (mean ICC 0.75 observer 1; mean ICC 0.66 observer 2).
Only 10% of all inter-examiner ICC’s comparing the results of examiners 1 and 2 shows significance levels below 0.05, except for the range of motion of the main axial rotation component. These findings are not related to one parameter or one mobilization technique.

Table 2: Intra-examiner Intraclass Correlation Coefficients for motion coupling parameters of three atlanto-axial mobilizing techniques: ICC (and significance)

A. Observer 1

	Observer 1					
Techniques	Regional		Fixation		Locking	
Parameters	ICC	*sign*	ICC	*sign*	ICC	*sign*
ROM X	**0.723**	**0.004**	**0.445**	**0.111**	**0.793**	**0.001**
ROM Y	**0.779**	**0.001**	**0.822**	**0.000**	**0.663**	**0.013**
ROM Z	**0.516**	**0.061**	**0.690**	**0.009**	**0.369**	**0.169**
CC	**0.552**	**0.044**	**0.548**	**0.051**	**0.558**	**0.046**
Ratio	**0.588**	**0.031**	**0.527**	**0,061**	**0.532**	**0.058**
Shift	**0.567**	**0.038**	**0.662**	**0.013**	**0.801**	**0.001**

B. Observer 2

	Observer 2					
Techniques	Regional		Fixation		Locking	
Parameters	ICC	*sign*	ICC	*sign*	ICC	*sign*
ROM X	**0.443**	**0.112**	**-0.352**	**0.736**	**0.309**	**0.221**
ROM Y	**0.710**	**0.005**	**0.621**	**0.023**	**0.670**	**0.012**
ROM Z	**0.156**	**0.358**	**-0.068**	**0.555**	**0.273**	**0.252**
CC	**0.327**	**0.198**	**0.073**	**0.437**	**-4.108**	**0.999**
Ratio	**0.776**	**0.001**	**0.400**	**0.144**	**0.825**	**0.000**
Shift	**0.095**	**0.415**	**-0.627**	**0.844**	**0.035**	**0.470**

ROM Z: range of flexion-extension motion component; *ROM Y*: range of main axial rotation component; *ROM X*: range of lateral bending motion component; *CC*: cross correlation coefficient; *Ratio*: ratio between axial rotation and lateral bending motion component; *Shift*: shift between coupled lateral bending component and main axial rotation motion component

ICC (Interclass Correlation Coefficient)<0 is 'poor', 0–0.20 'slight', 0.21–0.40 'fair', 0.41–0.60 'moderate', 0.61–0.80 'substantial' and 0.81–1.00 'almost perfect'

Significant values ($p<0.05$) are depicted in light azure field

Highly significant values ($p<0.01$) are depicted in dark azure field

Table 3: Inter-examiner Intraclass Correlation Coefficients for motion coupling parameters of three atlanto-axial mobilizating techniques: ICC values and significance

Technique	Regional							
Parameters	I		II		III		IV	
	ICC	*sign*	ICC	*sign*	ICC	*sign*	ICC	*sign*
ROM X	**0.460**	**0.094**	**-0.056**	**0.546**	**-0.201**	**0.653**	**0.272**	**0.248**
ROMY	**0.841**	**0.000**	**0.576**	**0.034**	**0.589**	**0.030**	**0.840**	**0.000**
ROM Z	**0.801**	**0.000**	**0.052**	**0.455**	**0.170**	**0.344**	**0.250**	**0.268**
CC	**0.326**	**0.199**	**0.647**	**0.014**	**-0.736**	**0.887**	**-0.161**	**0.626**
Ratio	**-0.110**	**0.589**	**-0.226**	**0.669**	**-0.083**	**0.568**	**-0.103**	**0.584**
Shift	**-0.087**	**0.571**	**-0.025**	**0.521**	**0.520**	**0.059**	**-0.698**	**0.871**

Technique	Fixation							
Parameters	I		II		III		IV	
	ICC	*sign*	ICC	*sign*	ICC	*sign*	ICC	*sign*
ROM X	**0.105**	**0.408**	**-0.430**	**0.779**	**-0.705**	**0.859**	**0.575**	**0.039**
ROMY	**0.802**	**0.001**	**0.701**	**0.006**	**0.571**	**0.045**	**0.792**	**0.001**
ROM Z	**0.574**	**0.039**	**-0.211**	**0.659**	**0.196**	**0.329**	**0.190**	**0.330**
CC	**-0.041**	**0.534**	**0.704**	**0.005**	**-0.185**	**0.635**	**-0.026**	**0.521**
Ratio	**-0.144**	**0.611**	**-0.453**	**0.789**	**0.019**	**0.484**	**0.287**	**0.240**
Shift	**-0.316**	**0.717**	**-0.121**	**0.597**	**0.427**	**0.131**	**-0.284**	**0.699**

Technique	Locking							
Parameters	I		II		III		IV	
	ICC	*sign*	ICC	*sign*	ICC	*sign*	ICC	*sign*
ROM X	**0.346**	**0.188**	**-0.126**	**0.601**	**0.064**	**0.446**	**0.303**	**0.226**
ROMY	**0.797**	**0.001**	**0.665**	**0.011**	**0.280**	**0.253**	**0.849**	**0.000**
ROM Z	**0.835**	**0.000**	**-0.053**	**0.544**	**-0.273**	**0.688**	**0.328**	**0.204**
CC	**-1.179**	**0.946**	**0.478**	**0.083**	**0.310**	**0.226**	**-0.767**	**0.882**
Ratio	**-0.075**	**0.560**	**-0.047**	**0.539**	**-0.143**	**0.607**	**0.074**	**0.436**
Shift	**0.157**	**0.361**	**-0.076**	**0.562**	**0.393**	**0.156**	**-0.458**	**0.784**

I: observer 1 test versus observer 2 test; II: observer 1 test versus observer 2 retest; III: observer 1 retest versus observer 2 test; IV: observer 1 retest versus observer 2 retest

ICC (inter class correlation coefficient)<0 is 'poor', 0–0.20 'slight', 0.21–0.40 'fair', 0.41–0.60 'moderate', 0.61–0.80 'substantial' and 0.81–1.00 'almost perfect'

Significant values ($p<0.05$) are depicted in light azure field
Highly significant values ($p<0.01$) are depicted in dark azure field

Due to the presence of large differences in the results from test and retest situations and between examiners, an analysis of variance (ANOVA) was performed for the mean data of the four test situations (T1-rT1-T2-rT2) comparing the three test situations. In this way, differences among these were found. ANOVA results showed significant differences among techniques for four parameters. The ROM of the coupled flexion-extension and CC values were not significant *(Table 4)*.

Table 4: ANOVA results reproducibility study between the mean of three atlanto-axial axial rotation mobilizing techniques.

Techniques	Regional	Fixation	Locking
Parameters	*sign*		
ROM Z	**0.270**		
ROM Y	**0.000**		
ROM X	**0.041**		
CC	**0.470**		
Ratio	**0.006**		
Shift	**0.000**		

ROM Z: range of flexion-extension motion component; *ROM Y*: range of main axial rotation component; *ROM X*: range of lateral bending motion component; *CC*: cross correlation coefficient; *Ratio*: ratio between axial rotation and lateral bending motion component; *Shift*: shift between coupled lateral bending component and main axial rotation motion component

Significant values ($p<0.05$) are depicted in light azure field

Highly significant values ($p<0.01$) are depicted in dark azure field

Subsequently, in order to verify exactly between which techniques there were significant differences for the four parameters, a Student's t-test for Paired Sample with Bonferoni adjustment (p=0.025) was executed. The following results were found *(Table 5)*: the ROM of the coupled lateral bending (ROM X) showed a significant relationship for the fixation-locking comparison inter-examiner; the ROM of the main axial rotation (ROM Y) showed a significant relationship for the regional-fixation and fixation-locking comparisons inter-examiner; both Ratio and Shift displayed significant relationships for the fixation-locking and locking-regional comparisons inter-examiner.

Table 5: t-test and significance levels for inter-observer comparison of the mean of three atlanto-axial axial rotation mobilizing techniques.

Techniques	Regional-Fixation		Fixation-Locking		Locking-Regional	
Parameters	t-value	*sign*	t-value	*sign*	t-value	*sign*
ROM X	**0.487**	**0.632**	**-2.737**	**0.013**	**2.079**	**0.051**
ROM Y	**6.871**	**0.000**	**-4.848**	**0.000**	**0.254**	**0.802**
Ratio	**2.461**	**0.024**	**-0.122**	**0.904**	**-2.968**	**0.008**
Shift	**-5.289**	**0.000**	**-1.715**	**0.103**	**5.677**	**0.000**

Significant values (Bonferoni adjusted $p<0.025$) are depicted in blue field

2.4 Discussion

A 3D kinematic analysis of unembalmed human spinal specimens in a test–retest situation with two observers is presented in this study. The purpose is to compare the results derived from the using of ZEBRIS ultrasound–based coordinate measuring system during manual mobilizations techniques at C1-C2 level, with other previous results obtained on embalmed specimens with the application of identical mobilization techniques. In this way, differences and correlations between these data can be highlighted.

In this study the total range of motion of the three angular motion components, were used together with the cross correlation, ratio and shift between axial rotation and lateral bending as objective parameters to describe motion coupling patterns.

Observing the results of the inter-observer comparison, an insufficient level of reproducibility for all three techniques performed is revealed for most of the analyzed parameters. The main axial rotation motion component (ROM Y) is the only parameter that shows significant inter-examiners and also intra-examiner reproducibility with "substantial" ICC-values in regional as well as in segmental fixation and locking techniques for both examiners.

The parameters describing the coupling patterns show only "moderate" to "substantial" intra-examiner reproducibility only for the examiners 1, the most experienced with the specific techniques applied in this study. All other correlations were not significant and no differences could be observed between regional versus segmental techniques.

The CC reflects the congruency between the Rom of the main axial rotation motion (ROM T) and the coupled lateral bending component (ROM X). The ANOVA results of reproducibility study for the three techniques indicate no significant differences between the data of test and retest of the two observers for this parameter (*Table 1*). This is probably due to low ICC for the CC in intra-tester and in inter-tester results. Indeed, these low values indicate that the correlation between the results of each couple of two datasets is low therefore, the ANOVA is unlikely indicate significant differences between different datasets. The CC show significant relationships only for the test–retest comparison of examiner 1 for the regional and locking techniques and for inter-tester correlation of the retests.

Observing the *Tables 2 and 3* it seems to be a general tendency towards higher intra-observer reliability compared to inter-observer results.

However, important differences are observed in intra-examiner reproducibility between examiners. There are contrasting opinions about the role of therapist background in the intra-observer reproducibility. Cattrysse et al. *(1997)* assigned to the experience a positive influence on the results and this appears confirmed by the present study that shows an higher reproducibility values for the first observer, more familiar with the examined techniques, in comparison with the second one which usually performed similar but not identical mobilizing techniques in daily clinical practice. So this tends to highlight that familiarization with the techniques might improve the reproducibility of the 3-dimensional kinematics of regional as well as segmental axial rotation mobilization of the atlanto-axial joint. However in observation of these results one should take in account the limitations inherent to the present possibilities for analyzing 3D kinematics of the atlanto-axial joint during manual mobilization.

In another *in vitro* study of Cattrysse et al. *(2007, J. of Electromyography and Kinesiology)* ten human spinal specimens (nine embalmed and one fresh) were examined by one investigator. The aim of this study was to analyse segmental motion coupling patterns at C1-C2 level during manually induced axial rotation and lateral bending. To do so, an electromagnetic tracking device - Flock of Birds - was used in combination with a 3D digitiser (Microscribe) which allows the registration of 3D features of an object in order to process the data mathematically. The results were related to bone embedded coordinate systems defined on the upper cervical spine segments, and the rotational components of coupled motion were analyzed.

In order to compare the results of this study with which of the present, only the data obtained from the analysis of the axial rotation mobilization at C1-C2 level are considered.
As the tests were performed by only one observer, just the intra-tester repeatability was evaluated.
The results of the axial rotation movements showed in 7 out of 9 embalmed specimens a controlateral coupling pattern between the main axial rotation and the coupled lateral bending. This was indicates by the presence of a negative CC (cross correlation) coefficient (-0.758; SD ± 0.164). The fresh specimen results revealed no significant difference from those of the 7 embalmed specimens, with the same coupling movement. Instead the other two embalmed specimens with ipsilateral coupling pattern showed a positive CC coefficient (0.888; SD ±0.121). The small SD values indicated an acceptable intra-tester reproducibility for CC parameter. A "moderate" reproducibility for this parameter also is showed in the present study on unembalmed specimens by the intra-examiner comparison of regional technique , but only for the observer 1.
The calculation of the Ratio shown for the controlateral specimens a mean value of 0.442 (SD ±0.222) and for the ipsilateral specimens a mean value of 0.432 (SD±0.169). These results indicated that during axial rotation mobilization the range of coupled lateral bending motion is somewhat less than half the main axial rotation motion and is slightly smaller in the specimens presenting ipsilateral coupling pattern. Also in this case the small values of SD indicated an acceptable intra-tester reproducibility for Ratio parameter. Also observing in the present study the results of intra-tester comparison of regional technique, a "moderate" reproducibility for observer 1 and an "substantial" for observer 2 for this parameter can be noticed.
In three specimens out of ten (two embalmed and one fresh) the test-retest procedure was executed. Z-scores were calculated for comparison of test and retest results and for comparison between the results of the fresh specimen with the results of the embalmed specimens. An acceptable degree of intra-examiner agreement for the three specimens tested was obtained. This was

indicated by the Z-scores < ± 2.0, meaning that the differences between test and retest motion components were within the limits of two standard deviation (SD). Considering the present study results on fresh specimens an high level of intra-tester reproducibility can be observed only in the observer 1, the most expert in the specific techniques.
Despite the difference in the structure between fresh and embalmed specimens also a good agreement (Z-scores < ±2.0) was indicated by the results of their comparison. Probably this is due to different amounts and moments of force applied on two type of specimens.
The mobilizations were executed 3 time consecutively.
The presence of small standard deviations per specimen for every range of motion of the main and coupled motions (SD between 0°and 4°) demonstrated an acceptable repeatability of manual mobilization. Also a good relationship for these parameters can be noticed in intra-examiner comparison for regional technique of tester 1.

Cattrysse et al. (2007 PhD ch. 1 part 2), in another *in vivo* study, analyzed the 3D kinematics of the upper cervical spine during manual mobilization techniques in axial rotation and in lateral bending. The effects of two different segmental manual techniques (manual fixation and locking of the inferior segment) were compared to those of regional mobilization techniques. The analysis was carried out by using the combination of FOB electromagnetic tracking device with a 3D digitiser (Microscribe).
The modality of analysis were very similar to the present study. However some important differences can be highlighted between them: the just depicted study includes one fresh and five embalmed human spinal specimens and the tests were executed only by one examiners; conversely the present study comprises only unembalmed specimens analyzed by two examiners.
In this discussion only data concerning the effects of axial rotation techniques at C1-C2 level are took in regard.

The results of the test-retest comparison for two specimens showed an acceptable degree of reproducibility for the main motion and the coupled motion, taking into account the limited number of specimens included and the small standard deviations. Also in the present study a good degree of reproducibility can be observed for all three techniques but only in observer 1.
The repeated measures ANOVA indicated significant differences between the three axial rotation situations: regional mobilization, segmental mobilization with manual fixation and segmental mobilization with locking of the inferior segments.
Statistical significance of the differences of mean range of motion of the main and coupled motions was indicated by a T-test with Bonferoni adjustment ($p<0.025$). The results shown that both locking and fixation segmental mobilization techniques, compared to so-called uni-planar regional mobilization, do not influence significantly the range of the main and coupled motion components during axial rotation mobilizations of the atlanto-axial joint. The manual fixation technique at this spinal level can significantly decrease only the involuntary flexion-extension coupled motion on the adjacent atlanto-occipital spinal motion segment.
Concerning the main axial rotation motion, in comparison with the regional mobilization the locking technique enabled an increase of this parameter, while the manual fixation technique restricted this main motion. However, according with Bonferoni adjustment($p<0.025$) these values are not significant.
These results can be compared with which of the present study on embalmed specimens. The results of the ANOVA performed for the mean data of the four test situations (T1-rT1-T2-rT2) to compare the three test situations, show significant differences among techniques for four parameters of the coupling motion. The following Student's t-test for Paired Sample with Bonferoni adjustment ($p<0.025$) indicates significant differences in the range of the main axial rotation and coupled lateral bending motions components between the two segmental mobilization techniques, and only in the range of main axial rotation between regional mobilization and fixation technique. Despite

some significant differences in parameters values can be noticed in all three comparison between mobilization techniques, major differences are between regional mobilization and fixation segmental technique.

Therefore, while in the study *(Cattrysse et al. 2007 PhD ch.1)* on embalmed specimens no significant influences on the range of the main and coupled motion components were found during axial rotation mobilizations with both segmental techniques compared with the regional, in the present analysis on fresh specimens significant differences for parameters of coupling motion were found among the three axial rotation situations, especially in regional mobilization-fixation technique comparison.

2.5 Conclusion

The results of in inter-observer comparison of this study show an insufficient level of reproducibility for all three techniques performed, for most of the analyzed parameters. Only the range of motion of the main axial rotation component shows a "substantial" level of intra- and inter-examiner reproducibility.

A general tendency towards higher intra-observer reliability compared to inter-observer may be observed. However, important differences also are present in intra-examiner reproducibility between examiners.

Comparing the results of the three *in vitro* studies a good agreement may be found concerning intra-tester reproducibility: despite the difference in the structure between fresh and embalmed specimens, both show acceptable values of reproducibility. However, concerning the fresh human specimens the parameters describing the coupling patterns show a "moderate" to "substantial" intra-examiner reproducibility mainly in regional mobilization and for only the examiner most experienced with the specific techniques of this study. This tends to indicate that familiarization with the techniques might influence the reproducibility of the 3-dimensional kinematics of regional as well as segmental axial rotation mobilization of the atlanto-axial joint, confirming the Cattrysse et al. study *(1997).* Anyway that should be interpreted considering the present limitations in the analysis of the 3D kinematics of manual induced motion of the atlanto-axial joint.

However the different intra-tester reproducibility between two observer with different background can not be also verified in embalmed specimens because of the presence of only one examiner.

Concerning the influences of the three mobilization techniques performed in this study on the parameters of coupling motion, some differences may be found between embalmed and unembalmed specimens. In the study *(Cattrysse et al. 2007 PhD ch.1)* on embalmed specimens no significant influences on the range of the main and coupled motion components were found during axial rotation mobilizations with both segmental techniques compared with the regional; on the contrary, in the present analysis on fresh specimens significant differences for parameters of coupling motion were found among the three axial rotation situations, especially in regional mobilization-fixation technique comparison.

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