TESIS DE LA UNIVERSIDAD
DE ZARAGOZA

2022

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Ana Isabel Lorente Corellano

Upper Cervical Spine Kinematics at the Intersegmental Level and the Role of the Alar Ligaments

Director/es
Maza Frechín, Marío Vicente
Hidalgo García, César

EXTRACTO

ISSN 2254-7606

Prensas de la Universidad
Universidad Zaragoza

ISSN 2254-7606

Tesis Doctoral [Extracto]

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Autor

Ana Isabel Lorente Corellano

Director/es

Maza Frechín, Marío Vicente
Hidalgo García, César

UNIVERSIDAD DE ZARAGOZA
Escuela de Doctorado

Programa de Doctorado en Ingeniería Biomédica

2022

Upper Cervical Spine Kinematics at the Intersegmental Level and the Role of the Alar Ligaments

Author

Ana I. Lorente Corellano

Supervisors

Dr. Mario Maza Frechín
Dr. César Hidalgo García

A dissertation presented in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Biomedical Engineering

April 2022

Acknowledgements

Regardless of how long this first paragraph is, there are not enough lines to thank both of my supervisors, Dr. Mario Maza Frechín and Dr. César Hidalgo García, for all of their support provided to me and my work during this PhD project. I have always been fascinated by interdisciplinary collaborations between Engineering and Health Science, and I could not have been feeling more grateful to be part of this collaboration linking Physical Therapy (César, *Dept. of Physiatry and Nursing*) and Engineering (Mario, *Dept. of Mechanical Engineering*). I would also like to thank Dr. Javier Marín Zurdo and José Manuel Auría Apilluelo (*IDERGO, I3A*), for their work during the early stage of this project and their overall support.

This PhD project demonstrates the first cooperation between the *Impact Laboratory (I3A; Univ. of Zaragoza)*, the *Physiotherapy Research Unit (School of Health Sciences; Univ. of Zaragoza)*, and *ACTIUM – Functional Anatomy Group (School of Medicine and Health Sciences; International University of Catalonia)*. I am grateful to Dr. Albert Pérez Bellmunt, Dr. Carlos López de Celis, and Dr. Jacobo Rodriguez Sanz (*ACTIUM*) for their work in the anatomical part of the in vitro tests. Currently, we are working on a second study, so I can already gladly say: I am looking forward to seeing what our second project brings to us and to Science!

I started this page mentioning my PhD supervisors, but I also want to thank (although they deserve a longer paragraph) my previous supervisors of research projects, prior to my PhD, including my periods at *KU Leuven* and *ENSAM*. Thanks to the work and time these supervisors shared with me, as well as their passion for science, I was clearly inspired to pursue a PhD.

There is one former supervisor who deserves a special recognition: Dr. Francisco J. López Valdés, as my first steps in science were under his supervision. Many research activities came during these years with Fran: my first conference presentation (in Philadelphia!) and my first experimental tests (including PMHS). I still have fond memories of all these years.

Another big THANKS is to Dr. Felix Stief, Dr. Stefan van Drongelen, and Jana Holder (*Orthopedics Clinic, University Hospital Frankfurt*). I am grateful for the way they welcomed me to their lab; working with the three of them is a pleasure. They made it possible for me to return home with lots of pleasant memories (and new knowledge), even with the COVID restrictions of that time...

I want to mention Óscar Juste (Impact Laboratory). Our research took different paths, but I will always remember the research moments we shared, along with Ana Piqueras, in the sled tests during the first years of my PhD.

Lastly, related as well with the Impact Laboratory, I am thankful for all the support of the Instituto Aragonés de Fomento (IAF, Gobierno de Aragón).

On a personal side, the first friend I want to mention is Wendy. I cannot express how special our weekly video-calls are. Wendy always has so much energy, I feel like we are close even though we live 9.000 km away from each other.

Luise, I appreciate all your support. After saying 9.000 km, Germany feels much closer now, but I wish it were closer after every single time we talk.

I want to include a general thanks to everyone who spent some time asking me about my PhD. Particularly: Alex, thanks for the countless times you wrote to me just to ask about the progress of this dissertation and for sharing your humor.

One group must appear here, and not only because they are part of my "engineering background" (as classmates during the Bachelor). Carlos, David, Fernando, and Dani, thanks for keeping our group alive and full of good humor and laughs.

Before thanking my family, let me say thanks as well to Hockey. Most of the time away from my desk has been spent in hockey rinks, playing this sport.

Mamá, Papá, if I started here my acknowledgments to both of you I would need to include the text of the dissertation in a second book. I will keep this part as short as possible: GRACIAS for all your support in all my academic pursuits.

Upper Cervical Spine Kinematics at the Intersegmental Level and the Role of the Alar Ligaments

Abstract

The upper cervical spine is often excluded from the kinematic studies of the cervical spine due to the specific morphology of its vertebrae: atlas (C1) and axis (C2). Widening the knowledge about the head-C2 biomechanics is of high clinical interest. Previous studies disagree with the maximum head-C2 mobility and how an alar ligament injury alters it. The alar ligament is a bilateral connection between the occipital bone and C2. Manual techniques in the upper cervical spine might imply a risk of neurovascular damage; to reduce this risk, the upper cervical spine stability is assessed with the mobility in pure lateral bending (side-bending stress test) and in axial rotation (rotation stress test). However, contradictions exist as to how an alar ligament injury alters the response during these manual tests. Furthermore, indirect mobilization approaches have shown good results in patients, e.g., improvement of C1-C2 mobility with C0-C1 mobilizations, but no biomechanical evidence supports these indirect approaches.

The objectives of this thesis were (1) to provide the intersegmental range of motion (ROM) of the upper cervical spine in lateral bending, axial rotation, and flexion-extension, (2) to analyze the effects of an alar ligament injury on the intersegmental mobility (with a unilateral ligament transection), (3) to assess the screening of this injury with in vitro manual tests (side-bending stress test and rotation stress test), and (4) to quantify how limiting the C0-C1 mobility influences the C1-C2 mobility.

Ten head-C2 specimens were manually mobilized in lateral bending, axial rotation, and flexion-extension in three conditions: (1) with intact alar ligaments, (2) with a C0-C1 screw stabilization, and (3) with a unilateral alar ligament transection. The motion was tracked with reflective markers and an optoelectronic capture system. To quantify the kinematics, a local coordinate system was created in each bone by measuring the coordinates of the reflective markers and the coordinates of anatomical landmarks. A load cell quantified the applied load through the full ROM.

These in vitro tests have reinforced the intersegmental ROM that can be expected in the upper cervical spine in a healthy population, consistent with previous in vitro and in vivo studies. The transection of one side of the alar ligaments has provided a better insight into which alterations can be expected when a patient has an alar ligament injury, and how these alterations might be detected with two manual clinical tests (side-bending stress test and rotation stress test) considering the ROM and the degree of stiffness. A bilateral increase of the ROM was quantified after the unilateral cut of the ligament, which is consistent with previous studies, but other studies had unilateral effects only on the contralateral side to a unilateral alar ligament cut. Lastly, related to the C0-C1 stabilization, the influence of limiting C0-C1 mobility has been seen in the axial rotation and flexion of C1-C2.

This thesis has provided new insights related to two clinical tests (side-bending and axial stress tests) and has deepened the understanding of the biomechanics behind the effect clinically observed in C1-C2 mobility with C0-C1 mobilizations.

Cinemática de la columna cervical superior a nivel intersegmentario y el rol de los ligamentos alares

Resumen

La columna cervical superior suele excluirse de los estudios sobre la cinemática de la columna cervical debido a la distinta morfología de sus vértebras: atlas (C1) y axis (C2). Sin embargo, ampliar el conocimiento sobre la biomecánica de la columna cervical superior es de un alto interés clínico. Hay estudios que discrepan sobre la máxima mobilidad entre la cabeza y C2, así como sobre cómo una lesión de los ligamentos alares afecta a dicha mobilidad. Los ligamentos alares conectan bilateralmente el hueso occipital con C2. Las terapias manuales aplicadas en la columna cervical superior pueden implicar un riesgo de daño neurovascular; para reducir este riesgo, la estabilidad de la columna cervical superior es evaluada mediante los tests de estabilidad cervical superior de inclinación lateral y de rotación. Sin embargo, existen contradicciones respecto a cómo una lesión en los ligamentos alares altera los resultados de estos test premanipulativos. Además, las técnicas manuales con un abordaje indirecto del segmento sintomático han mostrado buenos resultados en pacientes, como, por ejemplo, la mejora de la movilidad en C1-C2 con movilizaciones en C0-C1, pero faltan evidencias desde el punto de vista de la biomecánica que apoyen este abordaje indirecto.

Los objetivos de esta tesis han sido (1) aportar valores sobre el rango de movimiento (RDM) segmentario de la columna cervical superior en inclinación lateral, rotación axial y flexión-extensión, (2) analizar los efectos que puede provocar una lesión de los ligamentos alares en la movilidad segmentaria (con una transección unilateral en los ligamentos), (3) evaluar el diagnóstico de esta lesión con test manuales simulados en condiciones *in vitro* (test de estabilidad de inclinación cervical superior y test de estabilidad de rotación cervical superior), y (4) cuantificar cómo la hipomovílidad en C0-C1 puede limitar la movilidad en C1-C2.

Diez muestras anatómicas formadas por el cráneo, C1 y C2 fueron manualmente movilizadas en inclinación lateral, rotación y flexión-extensión en tres condiciones: (1) con los ligamentos alares intactos, (2) con una estabilización mediante tornillos en C0-C1, y (3) con una transección unilateral en el ligamento alar derecho. El movimiento se capturó con marcadores reflectantes y un sistema optoelectrónico. Para cuantificar la cinemática, se crearon sistemas de coordenadas locales en cada segmento midiendo las coordenadas de los marcadores reflectantes y las coordenadas de puntos anatómicos. Se usó una célula de carga para cuantificar la carga aplicada durante el rango de movilidad completo.

Estos ensayos *in vitro* han reforzado los valores en la literatura del RDM segmentario que puede ser esperado en la columna cervical superior en pacientes sanos, ya que los valores obtenidos son consistentes con estudios previos *in vitro* e *in vivo*. El corte unilateral del ligamento alar ha mejorado el conocimiento actual sobre qué tipos de alteraciones se pueden esperar en el RDM cuando un paciente tiene una lesión en los ligamentos alares, así como qué alteraciones serían probablemente detectadas en dos test de estabilidad cervical superior comúnmente realizados en el ámbito clínico considerando el RDM y la resistencia al movimiento durante la aplicación de los tests. Tras el corte unilateral del ligamento alar derecho, se cuantificó un aumento bilateral del RDM de manera consistente con estudios previos; aunque otros estudios anteriores habían detectado efectos unilaterales únicamente en el lado contralateral

al corte. Por último, en relación a la estabilización del segmento C0-C1, la limitación de movilidad en C0-C1 se vio reflejada en la rotación y en la flexión de C1-C2.

Esta tesis ha aportado nuevos conocimientos relacionados con dos tests de estabilidad cervical superior (de inclinación y de rotación) y han profundizado desde el punto de vista de la biomecánica sobre el efecto clínicamente observado en la movilidad de C1-C2 con la movilización de C0-C1.

Thesis by compendium of publications

This thesis is a compendium of the following **four accepted articles** in peer-reviewed research journals:

1. Hidalgo-García, C., Lorente, A.I., Rodríguez-Sanz, J., Tricás-Moreno, J.M., Simon, M., Maza-Frechín, M., Lopez-de-Celis, C., Krauss, J. and Pérez-Bellmunt, A., 2020. Effect of Alar Ligament Transection in Side-bending Stress Test: A Cadaveric Study. *Musculoskeletal Science and Practice*, 46, p.102110. https://doi.org/10.1016/j.msksp.2020.102110
   JCR Impact Factor: 2.520, Rehabilitation (Q2)

2. Hidalgo-García, C., Lorente, A. I., Lucha-López, O., Auría-Apilluelo, J. M., Malo-Urriés, M., Rodríguez-Sanz, J., López-de-Celis, C., Maza-Frechín, M., Krauss, J. and Pérez-Bellmunt, A., 2020. The Effect of Alar Ligament Transection on the Rotation Stress Test: A Cadaveric Study. *Clinical Biomechanics*, 80, p.105185.
   https://doi.org/10.1016/j.clinbiomech.2020.105185
   JCR Impact Factor: 2.063, Biomedical Engineering (Q3)

3. Lorente, A.I., Hidalgo-García, C., Rodríguez-Sanz, J., Maza-Frechín, M., Lopez-de-Celis, C. and Pérez-Bellmunt, A., 2021. Intersegmental Kinematics of the Upper Cervical Spine: Normal Range of Motion and its Alteration After Alar Ligament Transection. *Spine*, 46 (24), p.E1320.
   https://doi.org/10.1097/BRS.0000000000004167
   JCR Impact Factor (2020): 3.468, Orthopedics (Q1)

4. Hidalgo-García, C., Lorente, A.I., López-de-Celis, C., Lucha-López, O., Malo-Urriés, M., Rodríguez-Sanz, J., Maza-Frechín, M., Tricás-Moreno, J.M., Krauss, J. and Pérez-Bellmunt, A., 2021. Effects of Occipital-atlas Stabilization in the Upper Cervical Spine Kinematics: an in Vitro Study. *Scientific Reports*, 11(1), p.1-13. https://doi.org/10.1038/s41598-021-90052-6
   JCR Impact Factor (2020): 4.379, Multidisciplinary Sciences (Q1)

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Chapter 1

Introduction

This first chapter presents the background to better understand the work of this thesis entitled *Upper Cervical Spine Kinematics at the Intersegmental Level and the Role of the Alar Ligaments*. The first section shows the motivation for the research. Then, this chapter contains anatomy concepts related to the thesis, knowledge about assessing alar ligament injuries, and results of previous studies about cervical spine biomechanics. After this, the objectives of the thesis are enumerated, and at the end of this chapter there is an outline of the thesis.

1.1 Motivation

Neck pain is a common condition among adults (Hoy et al., 2010). Injuries in the upper part of the neck can cause instability and affect to neck mobility (Dvorak et al., 1988b; Antinnes et al., 1994; Debernardi et al., 2015). In physiological conditions, most of the neck's mobility occurs in the upper part of the neck, between the skull and the two uppermost cervical vertebrae, especially in axial rotation (White III and Panjabi, 1978; Zhang et al., 2020). Instability in this upper region can appear due to traumas, congenital conditions, or other scenarios, showing a wide range of mild symptoms, such as poor concentration and memory, nausea, tinnitus, headaches, and visual changes, but also life-threatening sequelae or even sudden death (Dvorak et al., 1988b; Corte and Neves, 2014; Henderson et al., 2020). Upper cervical spine instability is linked in many cases to damage in the alar ligaments, a bilateral structure which joints the occiput and the second uppermost vertebra, the axis. To better understand the biomechanics of upper cervical spine instabilities and how to assess them with manual mobilizations, the role of the alar ligaments needs to be clarified.

Manual mobilizations in the spine are common for the diagnosis and treatment of neck pain, mainly by physiotherapists, osteopaths, physicians, and chiropractors. But mobilization techniques are sometimes related to abrupt spontaneous movements, repeated mobilizations, or end-range rotations, which have a risk of severe complications in the vertebral arteries and internal carotid arteries (Hufnagel et al., 1999; Kerry et al., 2008; Ernst, 2007). The vertebral artery is fully stretched when the head is rotated within its physiological range of motion (Fielding, 1957). Therefore, instability of the upper cervical spine , and its increased mobility, could lead to mechanical stress in the cervical neurovascular system (Henderson et al., 2020). This artery is a vulnerable site for injury between the two uppermost vertebrae, and artery dissections have been reported causing cerebral ischemias, strokes, or deaths (Hufnagel et al., 1999; Kerry et al., 2008; Ernst, 2007; Hutting et al., 2018). Safety is a rising concern in spinal mobilizations whose goal is to improve the response of the upper cervical spine in the treatment of musculoskeletal dysfunctions.

To safely manipulate the upper cervical spine, practitioners consider expected ranges of motion. These values have been determined in previous studies for the three anatomical planes of motion: sagittal (flexion-extension), frontal (lateral bending), and transverse (axial rotation). However, different and wide ranges can be found in the literature, and an agreement considering inter-subject variability has not yet been reached (Bogduk and Mercer, 2000; Zhang et al., 2020). It has also been said that apart from the left and right range of motion, the asymmetry between both sides must be considered (Dvorak et al., 1987a). On the other hand, the validity of some manual tests for screening pathological upper cervical mobility must be established (Osmotherly et al., 2012; Rushton et al., 2014). For these reasons, new studies are valuable to better define the normal range of motion of the upper cervical spine, as well as to better know what mobility to expect in injury scenarios during manual screening tests for instability (Cattrysse et al., 2007a; Cattrysse et al., 2007b; Osmotherly et al., 2013a; Von et al., 2018).

Furthermore, related as well to manual mobilization, previous studies pointed out the possibility of improving the mobility of one spinal segment treating a different segment (Cleland et al., 2007). Improved mobility in the atlantoaxial joint has been proved without treating this segment directly and having only manual mobilizations one level above, in the occipito-atlantal joint (Hidalgo-García et al., 2016). This indirect approach of recovering C1-C2 mobility reduces the risk of neurovascular damage. However, the biomechanical reasons behind this clinical treatment are still unknown.

1.2 Anatomy of the Upper Cervical Vertebrae

The human spine is a flexible column with roles of vital importance, such as the stabilization of an upright position allowing mobility and protecting the spinal cord, enabling the connection with body organs. The spine is divided into three sections: cervical, thoracic, and lumbar (Oliver and Middleditch, 1991). The most cranial part is the cervical region and it is formed by seven vertebrae slightly curved with a lordotic shape, which means a posterior concavity (Galbusera and Wilke, 2018). The two most cranial vertebrae have a peculiar morphology, being the only two with specific names: **atlas and axis** (Figure 1.1).

The atlas is also named C1, following the standard nomenclature of the seven cervical vertebrae (from C1 to C7). This first vertebra connects the cervical spine to

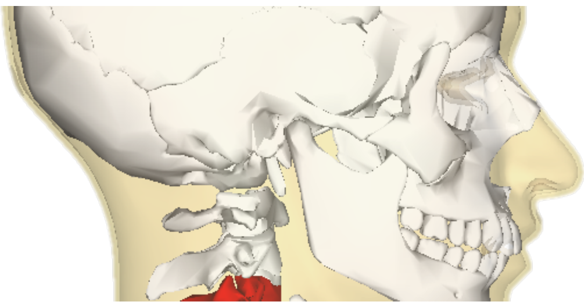

FIGURE 1.1: Skull, and under the occipital bone is the atlas and axis. The bone in red indicates the C3 level, the vertebra below the axis.

the occipital bone, which is the base of the skull. The occipital bone is sometimes named C0, following the C1-C7 nomenclature of the rest of the levels. In fact, the occipital bone is considered a part of the cervical spine in some biomechanical studies (Nahum and Melvin, 2012). Right below the atlas, the axis (C2) is located, which has a closer morphology to the rest of the cervical spine but has unique characteristics. Due to the differences found in the two most cranial vertebrae, they are named the **upper cervical spine**, while the rest (from C3 to C7) are known as the lower cervical spine and its morphology is more similar to the thoracic spine (Kapandji and Torres Lacomba, 2016). The morphological differences of the upper cervical spine are linked to its main functions: support of the head and head nodding and rotation (Olivetti, 2014).

1.2.1 Atlas

The main feature of the atlas is its shape: a ring shape with neither a solid cylindrical vertebral body nor any posterior element (spinous process) as in the rest of the vertebrae (Figure 1.2). The atlas, or C1, is formed only by two arches (anterior and posterior) linked through two lateral masses. The role of the anterior arch is the articulation with the vertebra below, while the posterior arch protects the spinal cord and brainstem (Nahum and Melvin, 2012). The bulkiest and solid parts of the atlas are the lateral masses, linked to their role of supporting the weight of the head (Gray et al., 1977). The name "atlas" describes the main role of this vertebra: the support of the head (Gray et al., 1977).

The joint between the skull and the atlas is called the **occipito-atlantal joint** and allows the head nodding thanks to its free sagittal plane movements. The C0-C1 joint is formed by the superior facets of the atlas and two bony protuberances on the base of the skull (occipital condyles). No cartilaginous structure is located at this level, as it occurs below C2 with the intervertebral discs. The main motion of this joint is flexion-extension (White III and Panjabi, 1978; Swartz et al., 2005). More about its kinematics can be found in Section 1.5, *Kinematics of the Upper Cervical Spine*.

FIGURE 1.2: The atlas (C1) has an unique shape in comparison with the rest of the vertebral levels. ⓪

1.2.2 Axis

The second uppermost vertebra, **axis** or C2, has this name because of being the pivot upon which the atlas rotates, while supporting the head (Gray et al., 1977). The most notorious difference for the axis is its conoid process growing upwards, named dens or odontoid process due to its tooth-like form (Gray et al., 1977). The internal structure of the odontoid process is more compact than the structure of any other vertebral body (Gray et al., 1977). Apart from the odontoid process and the vertebral body, the axis is also composed of pedicles, narrow and long laminae, short spinous process, flat articular processes, and short transverse processes (Gray et al., 1977). The dens reaches the posterior face of the atlas anterior arch to form the atlantoaxial joint (Figure 1.3).

FIGURE 1.3: Axis (C2) below atlas (C1). It is possible to see the dens of C2 behind the anterior arch of C1. ⓪

The articulation between these two uppermost vertebrae, the **atlantoaxial joint**, has a clear main difference, apart from the specific morphology, in comparison with the rest of the spine: the absence of an intervertebral disc. The intervertebral discs are between the rest of the vertebrae. They are fibrocartilage pads that separate vertebral bodies, allowing the bending and twisting of the spine (Newell et al., 2017). The atlantoaxial joint is formed by three synovial articulations. Two synovial articulations are the superior facet surfaces of the axis with the inferior facet surfaces of the atlas, and the third articulation is the anterior arch of the atlas with the dens of the axis (Nahum and Melvin, 2012). These are two different types of joints: the facet surfaces form gliding joints, while the anterior arch of the atlas is a pivot articulation (Gray et al., 1977). The posterior part of the dens is also in contact with the transverse ligament (Kapandji and Torres Lacomba, 2016). The articulation with the anterior arch of the atlas is located in the posterior face of the anterior arch, where the surface is smooth and oval in the area of the articulation (Gray et al., 1977).

The atlantoaxial joint allows our head to turn from one side to the other (Hamill and Knutzen, 2006), having in this cervical level the 50–55% of the axial rotation of the cervical spine (White III and Panjabi, 1978; Penning and Wilmink, 1987). It is the most mobile joint of all the cervical joints: it allows 10.0°–11.6° of flexion-extension ROM (White III and Panjabi, 1978; Frobin et al., 2002), and 37.8°–44.3° of axial rotation (Anderst et al., 2017; Dvorak et al., 1987a). Lateral bending has been described as not possible in this joint (White III and Panjabi, 1978), although other studies have found that some degrees of lateral bending can occur in the atlantoaxial joint (Ishii et al., 2006). More about its kinematics in the three anatomical planes can be found in Section 1.5, *Kinematics of the Upper Cervical Spine*

1.2.3 Vascular system

The vascular system has blood traveling adjacent to the upper cervical spine (Figure 1.4). At the level of the axis, the vertebral artery goes through the transverse processes; the transverse processes are perforated by the foramen for the vascular system (Gray et al., 1977). Regarding the atlas, in front of its posterior arch, behind each superior articular process, there is a groove where the artery runs (Hamill and Knutzen, 2006). At these levels (C1 and C2), the vertebral artery is particularly vulnerable to trauma (Oliver and Middleditch, 1991; Schievink, 2001). To follow the described path, the vertebral artery forms a serpentine course having multiple loops (Goel and Cacciola, 2011).

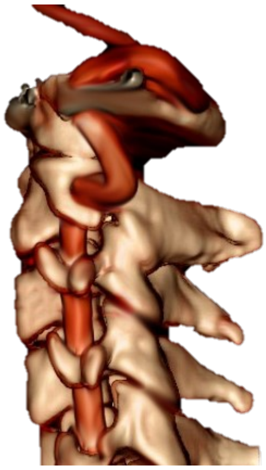

FIGURE 1.4: Vertebral artery at the level of the cervical spine. The serpentine path in the uppermost levels can be observed. ©

1.3 Anatomy of the Alar Ligaments

The alar ligament is formed by fibrous cords, which originate on the posterior lateral aspect of the odontoid process (C2) and arise bilaterally upward and outward with a "V" shape having their insertion in the occipital bone, the base of the skull (Gray et al., 1977; Panjabi et al., 1991c; Nahum and Melvin, 2012). The length of each side (left and right alar ligament) is 10.3 ± 2.0 mm (Panjabi et al., 1991c); and its cross-sectional area has been described as round, oval, or winglike by Lummel et al., 2010, but as rectangular (with a ratio of 2.6:1 and an area of 22 mm^2) by Dvorak et al., 1988b. The main role of the alar ligaments is the contribution to the **craniovertebral stability** (Dvorak et al., 1988b); the literature collects a wide range of possible morphological variations when trying to explain how the fibers of the ligaments stabilize the upper cervical spine (Cattrysse et al., 2007c; Sardi et al., 2017). This section summarizes different descriptions of the morphometry of the alar ligaments and their injury diagnoses.

1.3.1 Alar ligament attachments

In a wide number of research articles and anatomy textbooks, the alar ligament attachments are described as it was previously mentioned in this chapter: only as a connection between the dens and the occiput (Figure 1.5; Gray et al., 1977; Daniels et al., 1983). However, Cave, 1934, described the alar ligaments with a connection between the dens and the lateral mass of the atlas as well. According to Cave, 1934, these fibers are found in a similar plane as the fibers extended occipitally, and they are attached to the pretubercular recess of the atlas. This description of the ligaments has been lately reported by other studies. Dvorak et al., 1988b, defined the alar ligament as a two-portion structure: atlanto-alar and occipito-alar; the portion that connects the dens with the lateral mass of the atlas was found in all but one of the seven specimens examined. In other study by Dvorak and Panjabi, 1987, the presence of this atlantal portion was not found in several specimens: they conducted an anatomy study with 19 upper cervical spine specimens by taking CT images and the atlantal portion was identified in 12 out of the 19 specimens, and in the remaining 7 specimens the connection with the atlas was uncertain. Some years later, Panjabi et al., 1991c, worked with six human cadaveric cervical spines and also described the alar ligaments with a connection to the lateral masses of C1, apart from joining the dens and the occiput. Lastly, the description of having fibers inserted into the occipital condyles and, in a smaller portion, also into the atlas, can be found in anatomy books and review articles (Oliver and Middleditch, 1991; Debernardi et al., 2015).

On the other hand, other authors have specified that they were not able to find such connection with the atlas. Cattrysse et al., 2007c, did not find any ligamentous structure between the dens and the atlas in their 20 spinal specimens, although in all the specimens some soft connective tissue was localized in that area. Another case is the anatomical study of Osmotherly et al., 2013b, they examined by fine dissection 11 cervical spine specimens, and in none of them the connection with the atlas was found. More recently, Iwanaga et al., 2017a, detailed that they were unable to find any fiber of the alar ligaments linked to the atlas in any of their 11 specimens; they described these fibers as an anatomical variation. Lastly, it has also

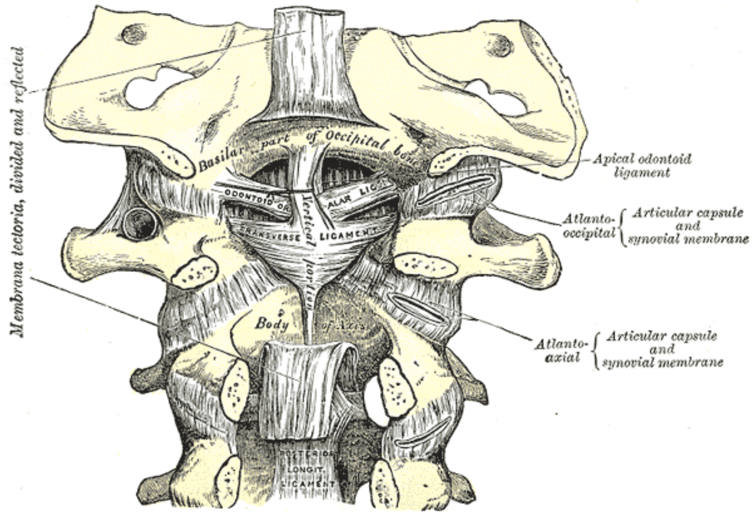

FIGURE 1.5: Alar ligaments (labeled on the right side of the ligament).©

been described in the literature the possibility that the alar ligaments can be without any attachment to the dens. Osmotherly et al., 2013b, found one case without any attachment to the dens in their 11 specimens studied by fine dissection.

Apart from the debate on the connection with the atlas found in the literature, the connection of the alar ligaments with the occiput is neither free of debate. Most of the studies indicate that the alar ligaments end only medially to the occipital condyles, close to the atlanto-occipital capsule (Althoff, 1979; Daniels et al., 1983; Dvorak et al., 1988b; Krakenes et al., 2001). But the cranial attachment has been also described at the anterolateral part of the foramen magnum (Tubbs et al., 2011).

1.3.2 Alar ligament orientation

Three different orientations have been described for the alar ligaments in different studies: caudocranial, horizontal, and craniocaudal. The orientation seems to have a relationship with the attachment point at the dens and the position of the occipital bone, which can be more or less cranial, as well as with the intersubject variability in bony morphology. Although this reasoning could seem right, and some research articles have proposed it (Dvorak et al., 1988b; Dvorak et al., 1988a), Osmotherly et al., 2011, proved that the height of the dens relative to the occipital condyles does not influence the ligament orientation.

The horizontal orientation in the frontal plane seems to be predominant. Dvorak et al., 1988b, found more often horizontally oriented ligaments, although some upward and downward ligaments were also detected. In the same line of these results are the studies of Okazaki, 1995, Krakenes et al., 2001, and Lummel et al., 2010. Okazaki, 1995, found in their 44 cadavers a total of 24 horizontal alar ligaments, 19 caudocranially, and only 1 craniocaudal case. And Krakenes et al., 2001, reported that 22 out of 30 volunteers studied by MRI had horizontal ligaments on coronal images, but other orientations were also seen: 5 cases had an upward orientation and the remaining 3 individuals had a downward orientation. Although the horizontal orientation was predominant in these studies, Pfirrmann et al., 2001, found these three possible orientations in a different proportion in their morphological analysis of the alar ligaments on 50 asymptomatic subjects: left and right sides were horizontal in 52% and 47%, respectively, of the cases, while 41% and 47% had them caudocranially, and it was craniocaudal in 7% and 5% of the subjects. Lummel et al., 2010, found these three orientations as well, but with the following percentages (in 50 volunteers): 58.5% were caudocraneal, 40.5% ran horizontally, and 1.0% had a craniocaudal orientation. Lastly, Panjabi et al., 1991c, described their six specimens with a caudocraneal orientation giving angles for the other two planes: 70° from the sagittal plane and 10° posterior to the frontal plane.

1.3.3 Histology

Histological studies of the alar ligament have been done by Saldinger et al., 1990, and also by Dvorak et al., 1988b, as part of a biomechanical analysis. The main structure of the alar ligament is formed by collagen fibers running parallel to each other and oriented as the main direction of the ligament, finding in the peripheral region a few elastic fibers (Saldinger et al., 1990; Dvorak et al., 1988b). It was seen that the fibers were denser packed on the dorsal side than in the ventral side (Saldinger et al., 1990). In the attachment of the ligament to the bones, Saldinger et al., 1990, observed a gradual transition from connective to bony tissue. Four zones were identified: ligament, fibrocartilage, mineralized fibrocartilage, and lamellar bone, as it was previously described by Cooper et al., 1970.

1.4 Evaluation of the Alar Ligaments

An objective criterion of injury for the soft-tissues of the cervical spine is needed in the assessment of patients. Due to difficult diagnostics, different image techniques for the examination of neck pain and focused on the alar ligaments have been discussed in the last decades. These techniques can be divided into two methodologies: direct and indirect. A **direct methodology** tries to see the status of the alar ligament through medical imaging, while an **indirect method** checks the alar ligament by quantifying the effect of a potential injury, such as any alterations in the range of motion of the upper cervical spine. All the indirect methods are based on the change of motion that an injured alar ligament is supposed to imply. The evaluation of the alar ligaments by manual techniques is discussed at the end of this section.

1.4.1 Image techniques

Starting with the studies focused on direct methodologies to see injuries on the alar ligaments, **magnetic resonance imaging (MRI)** is the most extended in use. Different anatomical structures of the upper cervical spine can be distinguished with the MRI technique (Schweitzer et al., 1992), and as particularly here concerns: the alar ligaments can be detected with MRI (Krakenes and Kaale, 2006; Kim et al., 2002; Schmidt et al., 2012).

By MRI, alar ligament injuries are seen as a high-signal change. In order to quantify injured ligaments, studies with healthy populations were done beforehand to establish how non-injured alar ligaments are seen on MRI images (Willauschus et al., 1995; Krakenes et al., 2001; Pfirrmann et al., 2001; Roy et al., 2004). An example of diagnostic use by MRI is with whiplash trauma patients. The alar ligaments are frequently damaged in rear-end impacts in car crashes (Kaale et al., 2005; Antinnes et al., 1994); and MRI techniques are used for diagnosis in whiplash-injured patients (Krakenes and Kaale, 2006; Wilmink and Patijn, 2001). Alar ligament injuries are usually graded according to the area of the ligament which registered a high signal. For example, Krakenes et al., 2001, ranked the grades between 0 and 3 as follow: the lowest (0) means low signal intensity throughout the entire cross-section of the ligament, and the rest of the grades mean a high signal intensity in one third or less of cross-section (1), in one third to two thirds of cross-section (2), and in two thirds or more of cross-section (3) (Krakenes and Kaale, 2006). Concerning the age of the patient and how this might influence the MRI ligament detection, Vetti et al., 2009, did not find a prevalence change with the age of the individuals (1,266 patients, age groups every 10 years, starting in younger than 20 and being above 60 in the last group). Nevertheless, degenerative changes due to age have been seen in MRI studies on other ligaments, also as high signal changes (Schweitzer et al., 1993).

Although the use of MRI is common in research studies about alar ligament injuries, it has the drawback of reporting high signal changes in non-injured individuals (Pfirrmann et al., 2001; Roy et al., 2004). This raises doubts about the real validity of MRI for detecting alar ligament injuries. Furthermore, the criterion of injury detection (high-signal change) was reported for other ligamentous structures and assumed to be also valid for the alar ligament by Krakenes et al., 2001, an extrapolation that has been questioned by other authors (Roy et al., 2004; Vetti et al., 2009). Finally, the imaging protocol has to be considered in the assessment of patients. One problem to properly detect the small alar ligaments might be the spatial resolution (slice thickness) or an insufficient contrast resolution (1.5, 2.0, 3.0 Tesla). A higher signal-to-noise ratio helps to better detect the alar ligaments (Schmidt et al., 2012). The lack of a standardized approach to determine the alar ligaments orientation has been criticized in the literature (Osmotherly et al., 2011).

MRI has been preferred over **computed tomography (CT)** by some authors because the soft-tissue contrast in CT may be insufficient to diagnose alar ligament injuries (Willauschus et al., 1995). In spite of this issue, research about alar ligament injuries and their direct visualization for diagnostic has also been done using CT (Daniels et al., 1983). Dvorak et al., 1987b, pointed out that by CT images, the alar ligaments can be visualized in coronal, sagittal, and axial images. It is always essential to decide on the methodology used according to the goal of what we want to see. For example, MRI would be better if we want to see the alar ligaments, but CT would be the right choice to see where the alar ligaments are attached to the bones due to the better bony definition in CT over MRI.

However, CT images have been more popular among the **indirect methods** in the assessment of alar ligaments. This means that by collecting CT images in neutral as well as in rotated position, the rotation is quantified and the alar ligament injuries can be predicted (Dvorak and Panjabi, 1987; Dvorak et al., 1987a; Antinnes et al., 1994). This indirect methodology is also known as **functional diagnostic**. Although MRI is used, as previously explained, for direct assessment of the alar ligaments, MRI can also be used to analyze the axial rotation range of motion, as usually done with CT (Osmotherly et al., 2013a).

Apart from CT, another method for indirect assessment of the alar ligaments is the **X-rays** images. X-rays do not provide direct information about the alar ligaments, but they are a good approach to study cervical spine stability. Lateral bending stability has been analyzed by Reich and Dvorak, 1986, with side-bending position X-rays by quantifying alar ligament laxity. This measurement was not done directly on alar ligaments but on the displacement of the atlas.

1.4.2 Manual therapy

Manual techniques to manipulate the cervical spine are accepted in assessment and screening protocols (Nordin et al., 2009; Gross et al., 2010). These manipulations might locate injuries leading to fewer and more specific MRI (Kaale et al., 2008). Furthermore, manual assessments have the benefit of providing clinical consequences of injuries, as a possible hyper-mobility, which would not be revealed on MRI (Kaale et al., 2008). It must also be considered that an alar ligament injury revealed on MRI might not necessarily cause upper cervical spine instability (Von et al., 2018). Manual therapy does not replace medical images assessment, which might be required to further evaluate patients (Kaale et al., 2008). Two clinical tests used to assess the integrity of the alar ligaments are the side-bending stress test and the axial rotation stress test. These two tests are performed prior to the application of manual therapy procedures.

The **side-bending bending stress test** consists of mobilizing the atlanto-occipital lateral bending through the head while the axis (C2) is stabilized to prevent its lateral bending and rotation (Aspinall, 1990). According to Kaltenborn, 2012, lateral bending with an effective C2 stabilization is only observed when the alar ligament presents laxity. A previous in vitro study reported an increase of 16.5% in lateral bending to the opposite side of an alar ligament transection, while showing only a 4.3% increase to the ipsilateral side of the ligament transection (Panjabi et al., 1991b). However, a later study reported a symmetrical increase in lateral bending after unilateral alar ligament transection (26% and 29%, Kettler et al., 2002).

The **rotation stress test** has the axis (C2) stabilized and the head is rotated to its maximum range of motion to assess the end-feel (resistance felt by the practitioner when the tissues reach maximum ROM) (Mintken et al., 2008). Moving the head in the transverse plane produces a greater effect on the contralateral side than the lateral stress test according to Osmotherly et al., 2012. Bilateral alterations in the rotation are expected after unilateral alar ligament injuries (Panjabi et al., 1991a; Kettler et al., 2002). An increase of 8.1% in axial rotation has been reported to the injured side, while this increase has been of 10.5% to the contralateral side (Panjabi et al., 1991a). Similar increases were reported by Kettler et al., 2002: 16% to the injured side and 12% to the contralateral side. One concern in the clinics related to this stress test is the total range of motion that must be expected: different studies have reported diverse ranges with differences up to 20° for the rotation stress test (Osmotherly et al., 2013a).

In both tests, the side-bending and the rotation stress tests, it remains unclear if the effects of an injury are detected only on one side or on both sides. Some authors have stated that the effects might be perceptible in only one side (Osmotherly et al., 2012). On the other hand, previous studies have pointed out that the effects in these manipulative tests are bilateral (Dvorak and Panjabi, 1987; Kettler et al., 2002).

The vertebral artery (Section 1.2.3) is vulnerable to trauma under certain manual manipulations (Oliver and Middleditch, 1991; Fabio, 1999; Schievink, 2001). The dissection of the vertebral artery can be triggered in spine manipulations by many other risk factors: recent head or neck trauma, migraine, recent infection, craniocervical vascular anomaly, etc. (Hutting et al., 2018). These two stress tests (side-bending and rotation) improve safety in manual mobilizations by identifying upper cervical instability (Osmotherly et al., 2012; Rushton et al., 2014). According to Osmotherly et al., 2012, both stress tests result in a measurable range of motion increase which can be manually felt, and both tests can be used in clinical practice. Nevertheless, the validity of these two stress tests must yet be established (Osmotherly et al., 2012; Rushton et al., 2014).

1.5 Kinematics of the Upper Cervical Spine

Kinematics describe motions between the bones, in this case between the occiput, atlas, and axis. These motions are influenced by the morphology of the bones and the joints between them; both aspects have been previously presented in Section 1.2, *Anatomy of the Upper Cervical Vertebrae*. The biomechanics of the cervical spine are very complex and have been the topic of countless research projects in the last decades aiming to improve the diagnoses and treatments of neck pain (Galbusera and Wilke, 2018; Panjabi et al., 1988). Neck pain is one of the most common musculoskeletal disorders and is frequently experienced in adult population (Vos et al., 2015).

The following three sections are divided in the three main planes in which the upper cervical spine can move: lateral bending in the frontal plane, axial rotation in the transverse plane, and flexion-extension in the sagittal plane. Each section describes the intersegmental kinematics: occiput-atlas and atlas-axis. Although the motions in the three planes are presented separately in different sections, head's pure movements are reached by mixed intersegmental movements out of the main plane. These effects on other planes are known as coupled motions (White III and Panjabi, 1978; Bogduk and Mercer, 2000; Ishii et al., 2006; Cook et al., 2006); and they have been also studied during manual mobilizations (Kapandji and Torres Lacomba, 2016; Cattrysse et al., 2008).

1.5.1 Lateral bending

No lateral bending occurs at the occipito-atlantal joint by the action of muscles, according to Fielding, 1957, White III and Panjabi, 1978, and Bogduk and Mercer, 2000. But Bogduk and Mercer, 2000, explained that lateral bending can be artificially produced by moving the head while manually fixing the atlas. However, lateral bending in the occipito-atlantal joint has been reported in healthy young volunteers without atlas fixation ($1.9 \pm 0.9°$, Ishii et al., 2006). In vitro studies have also reported lateral bending in this joint without atlas fixation: $5.6 \pm 3.0°$ (left side) and $5.1 \pm 2.5°$ (right side,Panjabi et al., 1991b). Other in vitro studies have reported similar values (Panjabi et al., 1988; Panjabi et al., 2001a); and the in vitro study of Goel et al., 1988, has also reported occipito-atlantal lateral bending ($3.4 \pm 2.8°$) even with a low torque (0.3 Nm).

At the atlantoaxial joint, although White III and Panjabi, 1978, have reported that no lateral bending is seen at this cervical level, other studies have quantified lateral bending at this joint. The in vitro study of Ishii et al., 2006, has reported a value of $1.6 \pm 1.3°$. In vitro studies have reported values up to $12.6 \pm 7.0°$ of lateral bending at the atlantoaxial joint (Goel et al., 1988; Panjabi et al., 1988; Panjabi et al., 1991b; Panjabi et al., 2001a; Zhang et al., 2020; Tisherman et al., 2020).

Other studies which reported lateral bending in both cervical levels are consistent with the idea that this motion can occur in the upper cervical spine (Penning, 1978; Kettler et al., 2002). Bilateral differences of 5.3° have been reported with no statistical significance (Panjabi et al., 1988; Dvorak et al., 1988a). Several studies have considered right and left sides symmetrical (Zhang et al., 2020).

1.5.2 Axial rotation

Some studies have stated that no axial rotation can happen at the occipito-atlantal joint, due to the contact of the atlantial sockets and the occipital condyles (Fielding, 1957; Penning, 1978; White III and Panjabi, 1978; Bogduk and Mercer, 2000). But Bogduk and Mercer, 2000, have described that occipito-atlantal rotation can be manually produced while fixing the atlas, and axial rotation has been in vivo measured: 1.7 ± 1.5° (Ishii et al., 2004) or 2.5 ± 1.0° (Salem et al., 2013), as unilateral values. Moreover, a higher value, 4.2 ± 1.8°, has been in vivo reported (Dvorak et al., 1987a). Values close to these measurements have been quantified following in vitro protocols (Panjabi et al., 1988; Panjabi et al., 1991a; Panjabi et al., 2001a; Dugailly et al., 2009; Zhang et al., 2020). In axial rotation, the C0-C1 segment has reported opposite motion to the C0-C2 direction, with -2° by Penning and Wilmink, 1987, or even -4°, by Iai et al., 1993, detecting this paradoxical counter-rotation in 30 out of 40 healthy men.

Axial rotation is the main motion at the atlantoaxial joint (Panjabi et al., 1988; Bogduk and Mercer, 2000; Panjabi et al., 2001a). One-side rotations up to 46.1 ± 12.5° have been in vitro measured (Dugailly et al., 2009); being very similar to the in vivo 44.3 ± 5.2° rotation measured (Dvorak et al., 1987a). Lower one-side rotations have been also quantified in other in vitro studies (31.4 ± 9.7°, with a range of 19.5°–50°, Dvorak et al., 1987b), as well as in other in vivo studies (36.2 ± 4.5°, Ishii et al., 2004, and 37.5 ± 6.0°, Salem et al., 2013). Other studies have reported one-side values between 34.0° and 40.5° (Penning and Wilmink, 1987; Panjabi et al., 1988; Panjabi et al., 1991b). Although the average values are similar, wide ranges have been reported, showing a high inter-individual variability: 29°–46° (Penning and Wilmink, 1987) or 22°–58° (White III and Panjabi, 1978).

The axial rotation of C0-C1 and C1-C2 might be closely related; an improvement in C0-C1 mobility has indirectly shown a better C1-C2 mobility (Hidalgo-García et al., 2016). Furthermore, bilateral differences between right and left axial rotation have been observed (7.2°), without being statistically significant (Panjabi et al., 1988). The differences in each segment between right and left axial rotation have been reported as 2.0° (0°–4°) in C0-C1 and 2.8° (1°–9°) in C1-C2 (Dvorak et al., 1987a). Several studies have considered right and left sides symmetrical (Penning and Wilmink, 1987; Boszczyk et al., 2012; Zhang et al., 2020).

1.5.3 Flexion and extension

Flexion-extension is the main motion at the occipito-atlantal joint (Bogduk and Mercer, 2000; Panjabi et al., 2001a). Flexion and extension motions are not symmetric (Fielding, 1957; Zhang et al., 2020). The range of motion at this joint for flexion can range between 7.2° and 14.4°, while for extension the values are larger, between 14.4° and 21.0° (Panjabi et al., 1988; Panjabi et al., 1991b; Panjabi et al., 2001a).

Flexion-extension is not the main motion one cervical level lower, at the atlantoaxial joint, but it can have a flexion range of motion between 7.7° and 12.7°, and a extension ranging from 10.5° to 12.1° (Panjabi et al., 1988; Panjabi et al., 1991b; Panjabi et al., 2001a; Anderst et al., 2015). Considering both levels, C0-C1 and C1-C2, Kettler et al., 2002, reported a flexion of 17.5° and a extension of 17.4°.

High inter-individual variability has been reported in flexion-extension range of motion. Considering both segments (C0-C2) ranges of 4°–33° (mean: 13°) for flexion and of 2°–21° (mean: 10°) for extension were reported in the review study by White III and Panjabi, 1978. A larger C0-C2 flexion-extension range of motion has been measured by Nightingale et al., 2007, in their in vitro study: 51.4 ± 9.3°. Other studies have reported lower values (C0-C1 + C1-C2): 14° + 13° (Lind et al., 1989) and 19.1° + 14.3° (Dugailly et al., 2009).

1.6 Biomechanical Role of the Alar Ligaments

The anatomical aspects of the alar ligaments and how to evaluate these ligaments in patients have been presented in Section 1.3 and Section 1.4. The kinematics of the upper cervical spine have been described in Section 1.5, and in the following paragraphs the role that the alar ligaments play on that kinematics is described. Although few articles have been published about the role that the alar ligaments have in the kinematics of the cervical spine, not all of the published studies are in agreement regarding the biomechanics of the alar ligaments and the unilateral or bilateral effects of its injuries.

Starting first without motion in the spine, just in a static neutral position, the first disagreement arises in the literature. The level of stress in the alar ligaments in neutral position is not clear: while Fick, 1904, pointed out that they are taught in this position, Werne, 1957, Dvorak and Panjabi, 1987, Panjabi et al., 1991a, and Crisco et al., 1991b described the alar ligaments as a lax structure at the neutral head position. Furthermore, the study of Crisco et al., 1991b, apart from describing the alar ligaments as lax when the head is at the mid-position, did a mathematical model which explains the rotation of the upper cervical spine in relationship with the stretching ratio of the alar ligaments. This model represents the first degrees of rotation at the atlanto-axial joint without ligamentous resistance.

The tensile strength of the alar ligament has been tested in several studies. A wide range has been provided, 87–346 N (mean: 186.9 ± 69.7 N), by Iwanaga et al., 2017b. A strength of 214 ± 69 N has been measured by Dvorak et al., 1988b, and 367 ± 83.2 N at a fast extension rate (920 mm/s) by Panjabi et al., 1998.

The morphology of the alar ligaments has been described as symmetrical (Iwanaga et al., 2017a), and also the rotation movement of the occipito-atlantal joint is symmetrical (Dvorak and Panjabi, 1987). Nevertheless, it can also be read in the literature that the occipito-atlantal joint can be asymmetric (Pfirrmann et al., 2001). The alar ligaments can limit the movement of the atlas indirectly by limiting the occiput-axis range of motion (Bogduk and Mercer, 2000). Furthermore, the alar ligaments have enough strength to restrain the anterior displacement of the atlas, which can be seen as a preventive mechanism against atlanto-axial subluxations (Fielding, 1957). But the most well-known role of the alar ligaments is to limit the axial rotation in the upper cervical spine (Fielding, 1957; Panjabi et al., 1991a).

When the head is rotated to one side, the contralateral alar ligament is tightened, which causes the traction of the occipital condyle on the contralateral side of the axial rotation (Fick, 1904; Werne, 1957). This traction of the occipital condyle causes a lateroflexion of the head to the opposite side of the axial rotation (Fick, 1904). In 1959, this combined movement was described due to the restriction by the both sides of the alar ligaments (Werne, 1959). Dvorak et al., 1987b, reported that in axial rotation, only the alar ligament of the opposite side is stretched; and showed that after unilateral alar ligament transection, only the axial rotation to the opposite side was increased (10.8° or 30%). By contrast, and using an in vitro setup as well, Panjabi et al., 1991a, described that after unilateral alar ligament transection the axial rotation was increased to both sides, being greater to the contralateral side. The increased range of motion was detected at both joints, C0-C1 and C1-C2: with a left side transection, the rotation to the left side increased 1.7° (51.5%, C0-C1) and 1.6° (4.3%, C1-C2), and the increases to the right side were 2.1° (35.0%, C0-C1) and 2.1° (6.2%, C1-C2,Panjabi et al., 1991a). Another study by Kettler et al., 2002, has also reported a symmetrical increase in axial rotation after left alar ligament transection: 3.9° higher to the left side and 3.0° higher to the right side.

In lateral bending, Panjabi et al., 1991b, and Kettler et al., 2002, reached different conclusions in their in vitro studies. Panjabi et al., 1991b, reported that after unilateral alar ligament transection, an increased range of motion in lateral bending was only observed to the contralateral side of the transection. In their study they cut the left alar ligament and quantified an increased lateral bending to the right side: 1.0° (19.6%) higher in C0-C1 and 1.3° (15.7%) in C1-C2. In the left lateral bending, the motion at C0-C1 decreased 0.2° (-3.6%) and increased 0.9° (7.1%) at C1-C2. In total, this was an increase of 0.8° (4.3%) to the ipsilateral side of the alar ligament cut (left side) and an increase of 2.3° (16.5%) to the contralateral side (right, Panjabi et al., 1991b). On the other hand, Kettler et al., 2002, reported a symmetrical increase: 2.1° to the contralateral side of the alar ligament cut and 2.2° to the ipsilateral side.

Lastly, in flexion-extension motion, the alar ligaments might play a greater role in flexion than in extension. Panjabi et al., 1991b, reported that after unilateral alar ligament transection a greater increase in flexion than in extension was observed. In both movements, flexion and extension, the increase was observed at C0-C1 and C1-C2 (flexion: 1.9°, 13.2%, at C0-C1, and 3.5°, 27.6%, at C1-C2; extension: 0.9°, 6.3%, at C0-C1, and 1.3°, 12.4%, at C1-C2, Panjabi et al., 1991b).

1.7 Objectives

This thesis has the following four objectives:

- To quantify the **intersegmental range of motion** of the upper cervical spine (occipital bone-atlas, atlas-axis) in the three anatomical planes: lateral bending, axial rotation, and flexion-extension.
- To analyze the **effects that an alar ligament injury might have** in the upper cervical spine kinematics (lateral bending, axial rotation, and flexion-extension).
- To validate the effects that a unilateral alar ligament transection have in the range of motion and stiffness during the **side-bending stress test and the rotation stress test**.
- To test how **hypomobility in the occipito-atlantal joint** might influence the kinematics of the atlantoaxial joint (in lateral bending, axial rotation, and flexion-extension).

1.8 Outline of the Thesis

After this introduction, the following four chapters correspond with the four research articles which form this thesis. The **Chapter 3** presents the C0-C2 kinematics in lateral bending and how unilateral alar ligament transection affects this motion. The paper therein presented simulates the clinical application of the side-bending stress test, which is a pre-manipulative screening test used to evaluate upper cervical instability. The **Chapter 4** presents the C0-C2 kinematics in axial rotation, and, as well as the previous chapter does, this chapter shows how unilateral alar ligament transection affects this motion, the axial rotation. This second paper simulates the clinical application of the rotation stress test. The **Chapter 5** shows the intersegmental kinematics of the craniovertebral junction (C0-C1 and C1-C2) in the three anatomical planes (lateral bending, axial rotation, and flexion-extension). This third paper collects data before and after unilateral alar ligament transection. Lastly, the **Chapter 6** reports how the occipito-atlantal joint mobility influences the kinematics of the atlantoaxial joint. This fourth paper considers the motion in the three anatomical planes.

After the four articles, the **Chapter 7** is the discussion chapter. Apart from summarizing the main findings of this thesis, the discussion chapter covers the following topics: experimental test setup to analyze in vitro spine kinematics, previous and current research about the upper cervical spine kinematics, and the clinical implications of this thesis. The strengths and limitations of this thesis are also presented in this chapter, as well as some future work lines.

Chapter 2

Material and Methods

This chapter describes the design of the in vitro tests and how all the tests were conducted. Head-C2 specimens were tested (Section 2.1), and both the anatomical and biomechanical procedures are herein detailed (Section 2.2 and Section 2.3). The measurements were conducted in the three anatomical planes (flexion-extension, lateral bending, and axial rotation) and in three different test conditions (before alar ligament transection, with occipital-atlas stabilization, and after alar ligament transection) (Section 2.4). Lastly, details about the evaluation of the results and the statistical analysis are provided (Section 2.5).

2.1 Sample

Ten head-cervical spine specimens from cryopreserved cadavers were prepared (9 males, 1 female; 74 years, range: 63–85). All the specimens were free of anatomical abnormalities and visually checked the day of the tests for evidence of any trauma that could influence the motion measurements. The specimens were also confirmed to be free of blood infectious diseases: hepatitis (B and C) and HIV.

The sample was obtained from the body donor program of the Universitat Internacional de Catalunya. The procedure herein described was approved by an ethics committee (Comitè d'Ètica de Recerca-Universitat Internacional de Catalunya; Ref. CBAS-2017-03).

2.2 Anatomical Procedures

The occipito-atlanto-axial specimens (C0-C1-C2) were obtained from cryopreserved cadavers which were stored at -14°C. This temperature of conservation keeps the biomechanical properties without significant alterations even for prolonged storage periods (Panjabi et al., 1985b). The specimens were thawed at room conditions (17.0–17.8°C and 47–52% of humidity) 24 hours prior to testing. These ranges of temperature and humidity for the room benefited the conservation of the specimens during the tests (Wilke et al., 1998a).

The specimens were obtained by disarticulating C2 from C3 through the intervertebral disc and zygapophysial facet joint capsules. All muscle tissues were removed while keeping the ligaments. The integrity of the atlas and axis was maintained. In order to create space for the motion capture sensors on C1, the mandible and the upper maxilla were removed. The posterior third of the skull had a wide wedge cut, which allowed the removal of the brain and the visualization of the foramen magnum (Dvorak et al., 1988b; Dvorak and Panjabi, 1987). Afterward, the brainstem, spinal cord, and dura were carefully removed to reach the right alar ligament.

Two more anatomical procedures are presented below to describe two tests conditions that were tested in the following order:

1. Occipital-atlas stabilization: this condition was achieved by a screw stabilization. Two self-tapping screws were introduced bilaterally 5 mm from the foramen magnum penetrating into the lateral mass of the atlas. Both screws in all the specimens were only partially threaded, remaining outside a portion of approximately 10 mm of the screws (Figure 2.1). The final position of both screws was visually assessed, and the segmental mobility

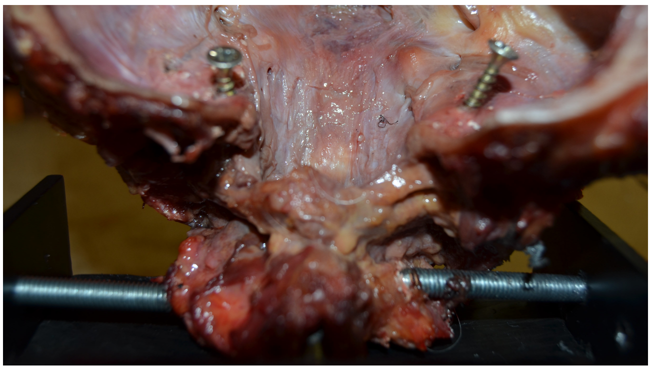

FIGURE 2.1: Occipital-atlas stabilization with two screws.

(occiput-atlas and atlas-axis) was checked after the placement of the two screws.

2. Alar ligament transection (right side): The alar ligament transection was through a 5–8 mm vertical incision on the tectorial membrane using a scalpel (surgical scalpel, handle number 3 and blade number 11). The vertical direction of the incision minimized tissue damages (Tisherman et al., 2020). Through this incision, the right alar ligament was delimited by palpation. Lastly, the complete dissection of the alar ligament was confirmed by using the tip of the scalpel to feel the bony surface of the anterior arch of the atlas (posterior aspect) throughout the full width of the right alar ligament (Figure 2.2). This procedure was facilitated by the fixed position of the axis and the skull; the axis was fixed on the load cell and the skull was supported in its neutral position by a metallic arm. Therefore, there was no motion between the segments during the alar ligament transection.

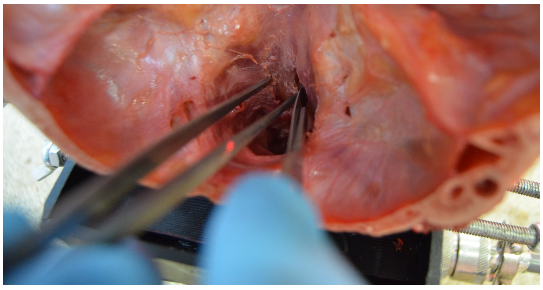

FIGURE 2.2: Unilateral transection of the alar ligament (right side).

To carefully follow this procedure a good knowledge of anatomy is needed, as well as the ability to perform anatomical dissections. All the anatomical preparations included in this project were done with great care to avoid dissecting deeper structures and by the same anatomist, who has more than 20 years of experience in anatomical dissections.

2.3 Biomechanical Procedures

Once the anatomical preparation was concluded with a specimen, a U-form metallic handlebar was attached to the skull by three points: both auditory canals and the top of the skull (Figure 2.3). This U-form handlebar was designed to move the skull manually during the tests without obscuring the sensors of the motion capture system from the cameras' view, as well as to avoid contacting these sensors while moving the skull.

The specimens with the handlebar were vertically oriented on C2 in its upright position (C2 below the head; Figure 2.3). The lowest level, C2, was fixed on a **six-axis load cell** (1.000 Hz; MC3-6-100 Force and Torque Sensor, Advanced Mechanical Technology Inc., Watertown, USA) to measure the required load to

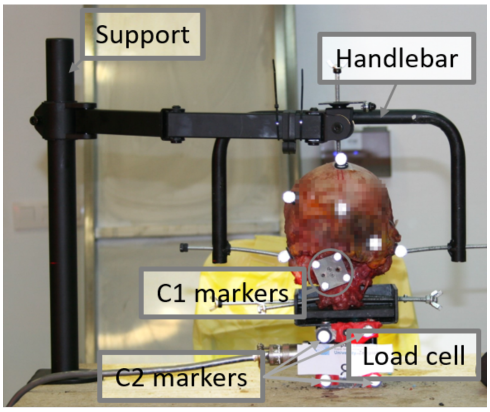

FIGURE 2.3: The experimental setup of the specimen attached to the load cell and with the Vicon markers and the mobilizing handlebar.

move the head. This load cell had a metallic support screwed on it, where C2 was fixed using five screws (two screws in each lateral side and one on the spinous process). The three anatomical planes of C2 were aligned with the three measurement axes of the load cell. Before the fixation of C2, the skull was oriented as in its neutral position (relaxed anatomical position when looking forward), ensuring that its neutral orientation was feasible once the C2 level was fixed.

The neutral position of the skull was ensured by two **red-light lasers**. One of the two lasers is shown in Figure 2.4. This laser was vertically oriented in front of the specimen's face. The specimens had the center of the face marked with a line passing through the chin, nose, and forehead, and this line was aligned with the laser. The second laser was horizontally oriented to match the anatomical Frankfurt plane. The reference known as the Frankfurt plane is defined with a line from the infraorbital foramen to the external auditory meatus (Moorrees and Kean, 1958). These vertical and horizontal references for the two lasers were used before starting each motion of the head in all the tests as the neutral reference.

The load cell measured continuously the load applied to the skull over the full load-cycle (from the neutral position to the end-position and back to the initial position). Each measurement was directly provided by the load cell in Newton-metre (Nm). Some of these measurements were converted to the applied force by the tester in the main plane of the motion (in Newtons, N). To obtain the forces from the measured torques the following two measurements were used. For flexion-extension and lateral bending movements the value was 130 mm: the height between the center of the hands of the tester and the estimated axis of rotation (mid-height of C2). For axial rotation the value was 150 mm: the half of the metallic handlebar width. The estimations of these two values were possible due to the small variability previously reported in the instantaneous centers of rotation within the segments of the cervical spine (Bogduk and Mercer, 2000; Amevo et al., 1991).

The motion was tracked with four cameras of a Vicon **motion capture system**

(1.000 Hz; TS Series, Vicon, Oxford, UK). These cameras emit infrared light, and the light is reflected back to the cameras by passive spherical markers. Two of the four cameras can be seen in Figure 2.4. Its own software Vicon Nexus was used. Firstly, each camera (focus and aperture) was adjusted, as well as other adjustments made on the software (such as the light that each camera sends and the bright threshold to accept a pixel seen by each camera). Afterward, the system was calibrated, allowing the system to know the position and orientation of all its cameras. The three-dimensional (3D) coordinates of each Vicon marker on the specimen are reconstructed by multiple 2D images from two or more cameras; at least two cameras need to track the same marker simultaneously. The error of the system for the measured angles was 0.0130°. The Vicon motion system has been previously used in other studies with cervical spine specimens (Yoganandan et al., 2007). The motion tracking was synchronized with the load cell with a manual trigger: both systems started simultaneously and recorded a pre-defined time of 15 or 20 seconds, depending on the movement.

FIGURE 2.4: Two Vicon cameras (of a total of four), and one red-light laser, which was used as the reference for the initial position (neutral position) before all the movements.

The spherical markers tracked by the Vicon cameras were attached to the bony structures as follows:

- Skull: six individual markers were attached directly to the skull on the external auditory meatus (bilaterally), inferior margin of the left orbit, centered on the top of the head and lower forehead, and laterally on the forehead. These six markers were placed on the head with glue (Loctite Super Glue-3, Henkel, Germany).
- Atlas: the available area on the atlas is small to place all the markers, therefore the markers were attached to the bone using a small metallic assembly. A metallic plate (10×15 mm) was screwed to the anterior arch with two parker screws of 8 mm, and another plate (35×35 mm) was connected therein with two standoffs. This second plate had four markers to measure the motion of the atlas (Figure 2.5). These plates were located so that there were no intersegmental motion alterations.
- Axis: this bone was rigidly attached to the load cell, therefore its markers were directly attached to the load cell.

The attachment of the markers to markers clusters fixed to the bone instead of having the markers directly on the bones has been previously validated by Lessley et al., 2011.

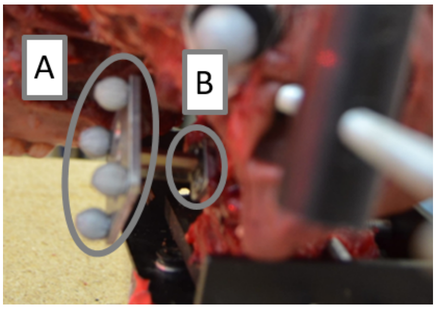

FIGURE 2.5: The markers of the vertebral level C1 (atlas) were attached to a metallic plate (A) which was assembled to a smaller metallic plate (B) fixed with two screws to the bone.

The motion of the markers was tracked by the Vicon cameras according to the laboratory's frame of reference. However, to know the motion with respect to anatomical coordinates systems it was required the transformations between the Vicon markers and the local coordinates systems. The 3D position of the markers were collected with a measuring device (FaroArm, FARO Technologies, Lake Mary, FL, USA), as well as the following anatomical landmarks of each bone:

- Skull: auditory meati (bilateral) and right infraorbital foraminae.
- Atlas: symmetrical right and left landmarks on the transverse processes, and anterior and posterior tubercles.
- Axis: symmetrical right and left landmarks on the transverse processes, lowest anterior central point on the body, and lowest central point on the spinous process.

The equations to define the local coordinates systems from these anatomical landmarks have been detailed by Slykhouse et al., 2019.

FIGURE 2.6: Measurement of the 3D coordinates of anatomical landmarks and Vicon markers with FaroArm to create the local coordinates systems of the skull, atlas, and axis.

Once the coordinates of the markers and the anatomical landmarks were measured, a matrix transformation between the markers and the local coordinates of the bones could be calculated following Kinzel et al., 1972. Shaw et al., 2009, have described this procedure and its equations in their article using also clusters of Vicon markers attached to bones. After determining the transformation matrix (markers-bone) with the coordinates of the measurement device (FaroArm), this matrix was applied to the data of the markers tracked by the Vicon cameras, and the local coordinates of the bone were known with respect to the Vicon coordinates system (Figure 2.7). After repeating the procedure of the transformation matrix with the three segments (skull, atlas, and axis), the intersegmental analysis of the motion was possible: the skull with respect to the axis, as well as the skull with respect to the atlas, and the atlas with respect to the axis.

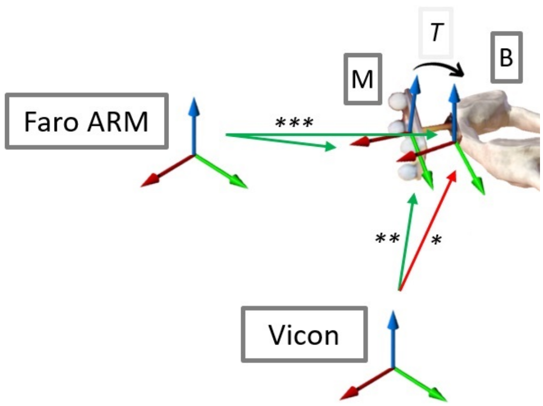

FIGURE 2.7: To know the local coordinate system of a bone with respect to the Vicon system (⋆) from the Vicon markers (⋆⋆), the matrix transformation (T) between the Vicon markers (M) and the bone (B) is needed and it can be calculated with the coordinate measurements of FaroArm. (⋆⋆⋆)

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2.4 Manual Mobilizations and Test Conditions

The motion on the skull was always applied manually from the posterior part of the specimen (Figure 2.3). The same sequence was followed in all the specimens for all the tests conditions:

1. Flexion-extension
2. Lateral bending
3. Axial rotation

The starting point in each mobilization was always the neutral position (a relaxed anatomical position when looking forward), which was checked with two red-light lasers as previously mentioned (Section 2.3). And the order of the test conditions was always the same:

1. Normal condition (before alar ligament transection)
2. Occipital-atlas stabilization
3. Alar ligament transection (right side)

Before starting to quantify the motion in each anatomical plane, a **warm-up procedure** was conducted: in the normal condition, for the three movements (flexion-extension, lateral bending, and axial rotation), the movements were repeated three times. The third time was recorded and the previous two cycles were as a preconditioning to reduce the influence of soft tissue viscoelasticity (Wilke et al., 1998a; Bernhardt et al., 1999). After tracking the three planes, the other two test conditions were done: occipital-atlas stabilization and alar ligament transection. The anatomical procedures to prepare these two test conditions are described in Section 2.2, *Anatomical Procedures*.

The movements were without pauses while a continuous rate was attempted. The rate in all the movements (for all the test conditions) was measured after conducting all the tests and it was always between 2.5°/s and 4.5°/s, which is within the recommended range of 0.5°/s and 5.0°/s (Wilke et al., 1998a). To achieve this low motion rate, the tester practiced the speed with a stopwatch before conducting the tests. The rate influences the response of specimens: a rate below 0.5°/s would introduce creep effects and a rate higher than 5.0°/s could lead to inertial effects (Wilke et al., 1998a; Panjabi et al., 1998).

The specimens were loaded until a firm end-feel was perceived by the tester, to simulate the clinical procedure (Osmotherly et al., 2012; Von et al., 2018; Kaltenborn, 2012). All the specimens were moved by the same practitioner. This person is a credentialed manual therapist and has more than 15 years of experience in orthopedic manual therapy treating patients with upper cervical impairments. The tester was blinded to the measured range of motion and load.

2.5 Evaluated Values and Statistical Analysis

The applied loads and their range of motions were continuously tracked. The results are provided for the maximum applied load as well as for other intermediate positions during the range of motion. These intermediate positions facilitated the comparison among the different specimens and the different tests conditions, as the ranges of motion under equal loads varied. The ranges of motion were compared on the following loads: 0.13, 0.26, 0.39, and 0.52 Nm (1, 2, 3, and 4 N) in flexion and extension; 0.26, 0.52, and 0.78 Nm (2, 4, and 6 N) in lateral bending; and 0.15, 0.30, and 0.45 Nm (1, 2, and 3 N) in axial rotation.

In the plots which describe range of motion and the applied load, the corridors show the average and standard deviation of all the specimens. The corridors were obtained following the method described by Lessley et al., 2004, which avoids discontinuities in the averaged curve due to the different range in the values of each specimen.

All the statistical analyses were done with the software SPSS (IBM SPSS Statistics for Windows, Version 23.0, IBM Corp. Armonk, NY, USA). The normal distribution of the sample was checked with the Shapiro-Wilk test. The differences between the normal test condition and the other two test conditions (occipital-atlas stabilization and alar ligament transected) were compared using the Wilcoxon rank test. The level of significance was set at 0.05.

Chapter 3

Effect of Alar Ligament Transection on the Side-bending Stress Test

The article presented in this chapter shows how alar ligament transection influences the lateral bending range of motion in the upper cervical spine. An increase in both sides, right and left, was observed after alar ligament transection: the initial lateral bending of $4.69 \pm 2.30°$ to the right side increased $1.30 \pm 1.54°$, and the initial $5.58 \pm 3.15°$ to the left side increased $1.88 \pm 1.51°$ after cutting the right side of the alar ligament, reaching $5.99 \pm 2.48°$ to the right side and $7.46 \pm 3.55°$ to the left side. Apart from these increases in the range of motion, greater lateral bending was observed after the ligament transection for certain load values (2 N, 4 N, and 6 N). The influence on the range of motion and the load could be decisive in clinical assessments using the side-bending stress test. The application of this screening test is discussed in the article.

Hidalgo-García, C., Lorente, A.I., Rodríguez-Sanz, J., Tricás-Moreno, J.M., Simon, M., Maza-Frechín, M., Lopez-de-Celis, C., Krauss, J. and Pérez-Bellmunt, A., 2020. Effect of Alar Ligament Transection in Side-bending Stress Test: A Cadaveric Study. Musculoskeletal Science and Practice,*46, p.102110.*
https://doi.org/10.1016/j.msksp.2020.102110

Musculoskeletal Science and Practice 46 (2020) 102110

Contents lists available at ScienceDirect

Musculoskeletal Science and Practice

journal homepage: www.elsevier.com/locate/msksp

Original article

Effect of alar ligament transection in side-bending stress test: A cadaveric study

César Hidalgo-García [a,*], Ana I. Lorente [b,1], Jacobo Rodríguez-Sanz [a], José Miguel Tricás-Moreno [a], Mathias Simon [c], Mario Maza-Frechín [b], Carlos Lopez-de-Celis [c,d], John Krauss [e], Albert Pérez-Bellmunt [c]

[a] *Researching Unit of Physiotherapy, University of Zaragoza, Spain*
[b] *Impact Laboratory, Aragón Institute of Engineering Research, University of Zaragoza, Spain*
[c] *Anatomy Unit, Department of Basic Areas, Universitat Internacional de Catalunya (Barcelona), Spain*
[d] *Fundació Institut Universitari per a la recerca a l'Atenció Primària de Salut Jordi Gol i Gurina, Barcelona, Spain*
[e] *School of Health Sciences, Oakland University, Rochester, MI, USA*

ARTICLE INFO

Keywords:
Alar ligament
Upper cervical spine
Physical therapy
Anatomy
In vitro
Manipulation

ABSTRACT

Background: The side-bending stress test is a pre-manipulative screening test for assessing upper cervical instability. To our knowledge, there is no study that simulates the clinical application of side bending stress test before and after alar ligament transection with fixation of C2.
Objective: To simulate the effect of alar ligament transection in the side bending stress test for an in vitro validation.
Design: In vitro study.
Methods: After the dissection of the superficial structures to the alar ligament and the fixation of C2, ten cryopreserved upper cervical spines were manually mobilized in right and left lateral flexion with and without right alar ligament transection. Upper cervical lateral flexion range of motion and mobilization force were measured with the Vicon motion capture system and a load cell respectively.
Results: The right alar ligament transection increased the upper cervical spine (UCS) range of motion (ROM) in both side bendings ($1.30°\pm1.54°$ and $1.88°\pm1.51°$ increase for right and left side bending respectively). As an average, with standardized forces of 2N, 4N and 6N, right alar ligament transection increased both right and left lateral flexion UCS ROM.
Conclusion: This in vitro study simulates the clinical application of the side bending stress test with intact and right transected alar ligament. Unilateral transection of the alar ligament revealed a predominantly bilateral increase in upper cervical side bending and variability in the mobilization force applied during the test.

1. Introduction

Manual therapists use cervical spine manipulation and mobilization to treat many different types of musculoskeletal dysfunctions (Carlesso et al., 2010; Gross et al., 2010). For the safe and effective practice of these techniques in the upper cervical spine, the identification of instability is fundamental. The stability of the craniocervical junction and the physiological mobility of the upper cervical spine in the frontal plane depend on the integrity of the alar ligament. This ligament is formed by two portions that connect the odontoid process to the lateral part of the foramen magnum of the skull.

According to Ishii et al. (2006), pure side bending of the cervical spine is accompanied by immediate ipsilateral rotation of the lower cervical spine (C2 and below) and contralateral rotation of the upper cervical spine (C0-C1, C1-C2). Dvorak and Panjabi (1987) suggested that coupled movements associated with cervical side bending are a direct consequence of alar ligament tension. Disruption of this bone-ligament-bone system (Crisco et al., 1991) may potentially increase the risk of neurovascular compromise in the upper cervical region during upper cervical rotation and side bending motions (Tubbs et al.,

* Corresponding author. Facultad de Ciencias de la Salud de la Universidad de Zaragoza, c/ Domingo Miral s/n, 50009, Zaragoza, Spain.
E-mail address: hidalgo@unizar.es (C. Hidalgo-García).
[1] equal contribution to the first author.

https://doi.org/10.1016/j.msksp.2020.102110
Received 10 August 2019; Received in revised form 14 December 2019; Accepted 13 January 2020
Available online 14 January 2020

2011).

Rushton et al. (2014) recommend the application of pre-manipulative screening before the use of spinal manipulation to identify any such disruption of the bone-ligament-bone system. They suggested that pre-manipulative testing include stabilization of the spinous process and lamina of C2 (to prevent both side bending and rotation of the segment) followed by a movement of the occiput into pure side bending. They theorized that if stabilization of C2 is effective, no upper cervical side bending will occur if the bone-ligament-bone system is intact. However, if motion occurs, then laxity of the alar ligament is suspected (Kaltenborn et al., 2012).

Osmotherly et al. (2012) measured the origin-insertion length of the alar ligament in vivo using MRI on 16 participants between the ages of 18 and 35. During their study, they compared the ligaments resting length with its length during pure side bending. They concluded that side bending causes an increase in length (a median between-side difference of 1.15 mm) of the contralateral alar ligament during side bending. Panjabi et al. (1991b) investigated the effect of the transection of unilateral and bilateral alar ligaments on upper cervical side bending in vitro. They identified a 16.5% increase in the contralateral side bending ROM following alar ligament transection in the first three cervical segments (2.3°) compared to nontransected specimens (13.9°±4.6°). Unlike the clinical side bending test where C2 is stabilized, Panjabi et al. fixated C3. The purpose of this study is to more accurately simulate the clinical side bending stress test by examining the effects of an intact and transected alar ligament on the side bending test in vitro with C2 fixated.

2. Materials and methods

2.1. Sample

Ten cervical spines and heads from cryopreserved cadavers (9 males and 1 female; mean age: 74 years, range: 63–85 years) were examined in this study. To determine suitability for inclusion, all specimens were visually checked for evidence of prior surgery, trauma, or any anatomical abnormalities that would influence ROM. In addition, all specimens were required to be free of any disease or contamination that would influence connective tissue integrity. All specimens were from a body donor program. The study was approved by a local ethical committee.

2.2. Anatomical and biomechanical procedure

All specimens were stored in a freezer at −14 °C and thawed to room temperature 24 h prior to data collection. To prepare each specimen, first, all spinal segments caudal to C2 were removed by disarticulating C2 from C3 by cutting through the C2-C3 intervertebral disc and zygapophysial facet joint capsules. Second, all muscle tissue in the specimens was removed with care taken not to disrupt any ligamentous tissues. Third, the cranial posterior third of the skull was removed using a wide posterior wedge cut as described by Dvorak et al. (1987, 1988) to allow for the brain to be removed and the visualization of the foramen magnum. Integrity of the posterior arch of atlas was maintained during this procedure. Forth, the brainstem, spinal cord, dura and part of the tectorial membrane were carefully removed to expose the alar ligament. Finally, for the purpose of attaching the measurement sensors the mandible and upper maxilla were removed. Once the specimen was ready for testing, a metallic handlebar was attached to the skull by three points, one through each auditory canal and one through the top of the head. The handlebar is pictured on Fig. 1, and it was designed to move the head without contacting any sensors attached to the specimens.

After the specimen was prepared, it was fixed to the load cell (MC3A Force and Torque Sensor, AMTI, MA, USA) which measured the force required to generate side bending of the head. More specifically, the C2 vertebra was screwed to a metallic support which was secured to the load cell. C2 was attached in the anatomical mid-position, aligned with the axes of the load cell. The head was aligned with the C2 position before each side bending motion was performed. To find the neutral head position, an anatomical Frankfurt horizontal plane was laterally marked on the head (through the external auditory meati and the infraorbital foraminae), a vertical line was also marked on the center of the face. These two lines were aligned with the horizontal and vertical references given by two red light lasers (previously calibrated to be horizontal and vertical). Head neutral position was checked before C2 was fixed to ensure that both were aligned in a neutral position after C2 fixation.

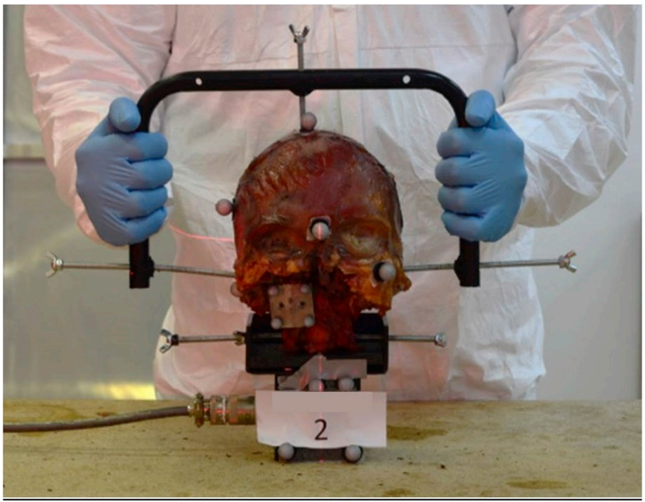

Fig. 1. Test setup: C0-C2 set up before mobilization in side bending once C2 has been fixated with screws, the handlebar has been fixated to the cranium and sensors have been attached.

An optical motion capture system (Vicon, TS series, Oxford, UK) consisting of four cameras was used to examine the 3D motion of the head over C2 during side bending. Retroreflective spherical markers placed on the head and C2 (Fig. 1) were used to define head and C2 coordinate systems. Each specimen was side bent four times. The first two side bending motions were used as a warm-up to reduce the influence of soft tissue viscoelasticity (Wilke et al., 1998), with all measurements being recorded on the third motion (alar ligament intact). After transecting the right alar ligament, side bending was once more registered. To simulate the passive side bending stress test, all movements were induced manually in the frontal plane between 0.5 and 5.0°/s as recommended by Wilke et al. (1998). The movement ended when the researcher perceived a marked resistance. All side bending movements for all specimens were performed by the same researcher with more than 15 years of experience in manual therapy. To prevent dehydration and ensure that specimens remained physiologically viable, the room temperature was maintained between 17.0° and 17.8° Celsius and the humidity was maintained between the 47–52%.

SPSS statistical software (version 20.0) for Windows was used for all statistical analyses. A descriptive analysis of side bending angles and forces applied during the experiment was performed. The differences between normal and transected specimens was compared using the Wilcoxon rank test. Shapiro-Wilk test was used to identify the normal distribution of the sample. The level of significance was set at alpha = 0.05. The average and standard deviation corridors of the results from the 10 specimens were obtained following the method described by Lessley et al. (2004). This method avoided discontinuities in the averaged curve due to the different ranges of results between the specimens.

3. Results

Fig. 2 illustrates the amount of force applied and the resultant side bending movement for all ten specimens with both alar ligaments intact (illustrated in black) and with the right alar ligament cut (illustrated in grey). When reading the graphs, positive flexion values indicate right side bending, and negative values indicate left side bending. Table 1 contains the side bending angles recorded for each specimen (normal and transected) when the applied forces were 2N, 4N, and 6N, as well as the force applied to achieve maximum ROM. Table 2 shows the comparison of the side bending angles and associated forces for each specimen (normal and transected).

During right side bending, all specimens demonstrate an increase in side bending angles following the transection of the right alar ligament except for specimen 2 (1.30°±1.54° as total average in the end range). The average increase of ROM in right side bending with right alar ligament transection achieved statistical signification at the end-range *($p < 0.01$)*. This is illustrated in Fig. 2, where the grey line mostly shows higher or equal values to the black one for a given force value. The average increase of ROM ranged between 0.78° at 4N and 1.53° at 6N. Only the average increase with right alar ligament transection, at the standardized force of 6N, achieved statistical signification *($p < 0.04$)*.

During left side bending, all specimens demonstrated an increase in side bending angles with the transection of the right alar ligament except for specimen 4 (1.88°±1.51°) as a total average at the end range. The average increase of ROM in left side bending with right alar ligament transection achieved statistical signification at the end-range *($p < 0.01$)*. Specimen 4 showed a reduction of - 0.74° at the end range, although the force used to achieve end range was 4.03 N lower after the alar ligament had been cut as compared to when it was intact. This phenomenon was not the same throughout the specimens: specimens 1, 2, 8, 9 and 10 had an increase in the angles for the same force examined when comparing with the normal specimens, but specimens 3, 5 and 7 showed a decreased angle after the transection at lower force values (2N and 4N) but not with higher force values (6N and end range). For specimens 4 and 6, the left side bending showed similar values at 2N, 4N, and 6N. Only the increase average with right alar ligament transection at the standardized force of 6N achieved statistical signification *($p < 0.028$)*. The average of the 10 specimens with standard deviation is showed in Fig. 3, for intact condition (black line) and for the right alar ligament cut (grey line).

4. Discussion

The side bending stress test reportedly examines the stability of the upper cervical spine by checking the function of the alar ligament. The assumption is that if the alar ligament is intact, there will be little to no side bending of the occiput possible during the test. To examine this assumption, specimens were laterally flexed before and after the transection of the alar ligament, and the amount of motion and force required to generate maximal side bending was measured.

To our knowledge, this is the first biomechanical study that simulates the clinical application of the side bending stress test before and after alar ligament transection. The results of this study show that unilateral transection of the alar ligament results in a mostly bilateral increase of the side bending ROM. Also, when standardizing the force of mobilization at 6N, there was a statistically significant bilateral increase in the side bending ROM.

4.1. Range of motion in side bending stress test

The upper cervical side bending ranges measured in this study (4.69° to the right and 5.58° to the left) are slightly lower than the in vitro estimates (7.9°–12°) of movement that have been reported in previous literature (Panjabi et al., 2001) and slightly higher than the in vivo measurements (3.3°) (Ishii et al., 2006). While these studies used C3 or below as the fixation during side bending, this study used stabilization of C2. This distinction is important because when C2 is not fixated, side bending of the head is accompanied by immediate ipsilateral rotation of the C2 beneath C1 (Osmotherly et al., 2012) which likely leads to the increase in side bending reported by Panjabi et al. When C2 is fixated, the odontoid and C2 cannot shift laterally or rotate and tension is immediately increased in the contralateral alar ligament during side bending. Thus, if C2 is fixated a reduction in side bending is expected. In addition, the use of Frankfurt may have provided a consistent neutral reference and allowed a more precise measurement than those of prior authors. The fact that any degree of motion is present when C2 is fixated supports the conclusion from Dvorak and Panjabi (1987) that the alar ligaments are not tight in the mid position. The presence of upper cervical side bending, although very limited, in this vitro study also challenges the assumptions associated with stability testing of the alar ligament (Pettman, 1994; Kaltenborn et al., 2012; Beeton, 1995) that no

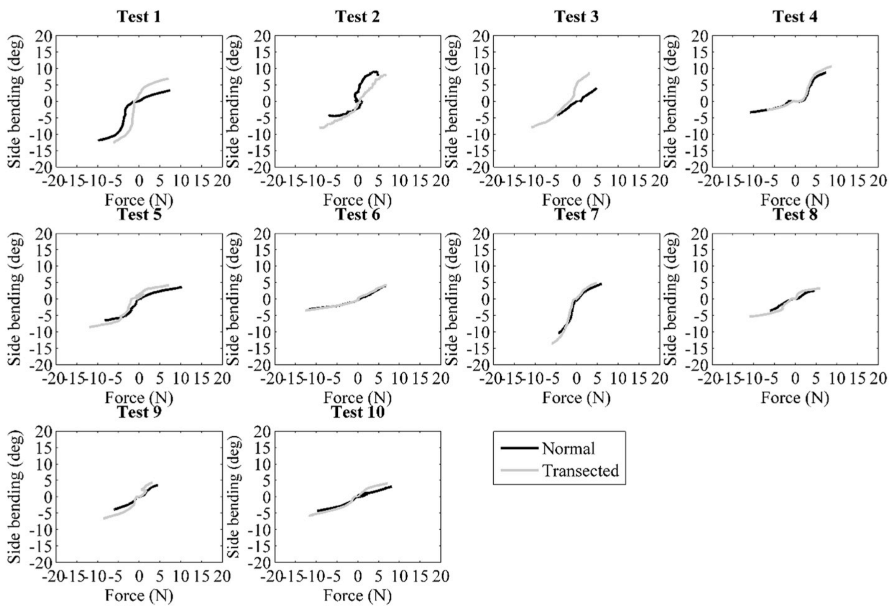

Fig. 2. Forces required for side bending during the full range of motion in the 10 specimens: normal and transected.

Table 1
Outcomes of the side bending angles for each specimen at 2, 4, and 6 N, the maximum force applied (F. Max) and the maximum range of motion (ROM Max).

Test	N/T	Force									
		Right side bending					Left side bending				
		2 N	4 N	6 N	F. Max	ROM Max	2 N	4 N	6 N	F. Max	ROM Max
1	**Normal**	1.46	2.27	2.89	7.39	3.37	0.71	7.23	10.44	9.85	12.07
	Transected	4.81	5.86	6.63	7.08	6.89	7.79	10.84	12.48	6.24	12.60
	Difference	3.35	3.59	3.74	−0.31	3.52	7.08	3.62	2.04	−3.61	0.53
2	**Normal**	7.68	8.94		4.80	9.02	3.20	4.18	4.44	6.96	4.46
	Transected	2.76	5.36	7.89	6.69	8.62	3.59	5.42	6.56	9.25	8.10
	Difference	−4.92	−3.57		1.89	−0.40	0.40	1.24	2.12	2.29	3.64
3	**Normal**	1.52	3.20		4.75	4.05	1.93	3.69		4.75	4.14
	Transected	7.46			3.13	8.48	0.31	2.95	5.24	10.89	7.90
	Difference	5.95			−1.62	4.43	−1.62	−0.74		6.14	3.76
4	**Normal**	0.34	5.40	8.12	7.36	8.83	0.49	1.82	2.47	10.89	3.41
	Transected	0.70	6.92	9.20	8.72	10.63	1.04	1.94	2.47	6.86	2.67
	Difference	0.36	1.52	1.08	1.36	1.80	0.55	0.11	0.00	−4.03	−0.74
5	**Normal**	1.25	2.08	2.68	10.25	3.69	3.29	5.42	5.42	8.26	6.47
	Transected	3.07	3.53	3.96	7.18	4.34	0.61	4.96	7.00	12.01	8.56
	Difference	1.82	1.45	1.28	−3.07	0.65	−2.68	−0.46	1.58	3.75	2.09
6	**Normal**	0.87	2.30	3.76	6.66	4.13	1.10	1.82	2.25	11.76	3.15
	Transected	1.32	2.54	3.80	6.87	4.31	1.17	1.81	2.27	12.67	3.46
	Difference	0.45	0.25	0.04	0.22	0.18	0.07	−0.01	0.02	0.91	0.31
7	**Normal**	2.22	3.63		5.98	4.63	7.06	10.02		4.28	10.41
	Transected	2.99	4.49		4.80	4.87	5.88	11.54		5.92	13.64
	Difference	0.77	0.85		−1.18	0.24	−1.18	1.52		1.63	3.23
8	**Normal**	1.47	2.41		4.65	2.57	0.66	2.29	3.50	6.04	3.50
	Transected	2.47	2.92		5.95	3.31	1.10	3.58	4.32	11.06	5.34
	Difference	1.00	0.50		1.30	0.74	0.44	1.29	0.83	5.02	1.84
9	**Normal**	1.87	3.38		4.45	3.55	1.91	2.99		5.98	3.87
	Transected	3.61			3.19	4.36	3.20	4.80		8.59	6.61
	Difference	1.74			−1.26	0.82	1.29	1.81		2.61	2.73
10	**Normal**	1.22	1.57	2.27	8.13	3.09	1.61	2.74	3.37	9.79	4.33
	Transected	2.34	3.24	3.77	7.12	4.13	1.93	3.21	3.97	11.75	5.73
	Difference	1.13	1.66	1.50	−1.01	1.04	0.32	0.47	0.61	1.97	1.40

Abbreviations: N: Newtons; F: Force; Max: Maximum; ROM: Range of Motion.

Table 2
Comparison between Normal and Transected outcomes of the side bending angles with the different forces, and maximum force applied and the maximum range of motion.

	Force									
	Right side bending					Left side bending				
	2 N	4 N	6 N	F. Max	ROM Max	2 N	4 N	6 N	F. Max	ROM Max
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Normal	1.99 ± 2.06	3.52 ± 2.19	3.94 ± 2.40	6.44 ± 1.89	4.69 ± 2.30	2.20 ± 1.97	4.22 ± 2.65	4.55 ± 2.82	7.86 ± 2.63	5.58 ± 3.15
Transected	3.15 ± 1.89	4.36 ± 1.56	5.88 ± 2.37	6.07 ± 1.82	5.99 ± 2.48	2.66 ± 2.48	5.11 ± 3.43	5.54 ± 3.28	9.52 ± 2.52	7.46 ± 3.55
Differences	1.17 ± 2.72	0.78 ± 2.03	1.53 ± 1.36	−0.37 ± 1.56	1.30 ± 1.54	0.47 ± 2.62	0.89 ± 1.29	1.03 ± 0.89	1.67 ± 3.30	1.88 ± 1.51
p-value	0,059	0,123	**0,043***	0,646	**0,013***	0,646	0,059	**0,028***	0,169	**0,013***

Abbreviations: N: Newtons; F: Force; Max: Maximum; ROM: Range of Motion; SD: Standard Deviation; *statistical significance (Wilcoxon test).

pure side bending is possible with fixation of C2 if the ligaments are intact.

It has traditionally been assumed that upper cervical side bending is limited by the contralateral alar ligament. Panjabi et al. (1991b) obtained an increase (16.5% of the initial range of motion) only in contralateral side bending and not in ipsilateral side bending (4.3% of the initial range of motion) after the unilateral transection of an alar ligament. Osmotherly et al. (2012) also supported the notion that the contralateral alar ligament limited side bending during the side bending stress test using MRI measurements on 16 asymptomatic participants. In contrast, the present study revealed an increase in upper cervical ipsilateral side bending (33.5%) and contralateral side bending (27.5%) after the unilateral transection of an alar ligament. The results of this study supports the model of Crisco et al. (1991), which predicts that the rupture of one alar ligament impacts the stability of the other alar ligament and will lead to an increase in side bending in both directions. However, these results should be considered with caution when extrapolating into clinical practice. The increase of ROM in side bending was not present in all the specimens. So, potentially, the side bending stress test would not be able to detect ligament damage in all our specimens. Also, based on the low average increase of side bending (1.3–1.88°) found in this study, it is questionable if the practitioner would be able to detect a change in motion during the side bending stress test in a clinical setting.

4.2. Resistance during side bending stress test

This study also measured the amount of resistance to side bending before and after the transection of the right alar ligament. The study found variability in the amount mobilization force required to generate left and right side bending in the physiological ROM (same ROM produced with normal alar ligament). In most of the specimens, more ROM in side bending was produced with the same force following unilateral alar ligament dissection. In a few specimens, when comparing the amount of motion at standardized lower forces (2N, 4N), some of the transected specimens showed less side bending ROM, however, at higher

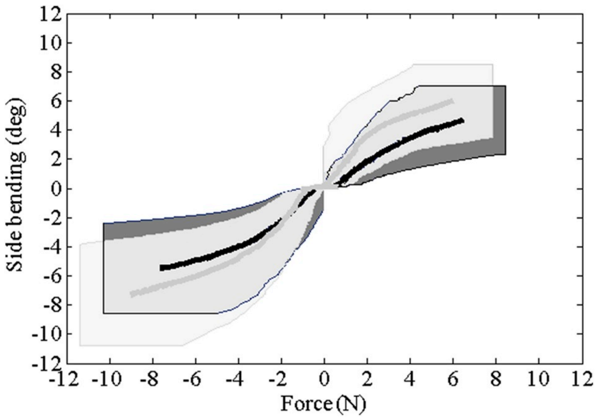

Fig. 3. Average side bending of the 10 specimens, for intact condition (black line) and for the transected right alar ligament (grey line). Their standard deviation corridor is plotted in dark grey and light grey respectively, being the common values of both conditions in a middle grey tone.

forces, these same specimens demonstrated increased motion as expected. Increases in ROM combined with reduced resistance to motion have been described as a key indicator of instability (Panjabi et al., 1991a).

From a clinical perspective, it would seem reasonable that the resistance perceived during passive side bending should be considered when diagnosing upper cervical instability since there appears to be a large variation in motion within the region (Lummel et al., 2012). Kaale et al. (2008) supported this conclusion by demonstrating it is possible to detect joint hypermobility and ligament injury in the upper cervical spine by a clinical examination based on an assessment of the quality of occipito-atlanto-axial rotation performed by one examiner, compared to ligament damage diagnosed by MRI, in patients with chronic whiplash. Some of the specimens in this study demonstrated a decrease in resistance in the unstable ROM (beyond the range produced with the alar ligament intact). Whether a clinician can detect an alteration in the quality of movement throughout the range of movement including the unstable range is unclear. The increase in the resistance at the end range for a few specimens could have been produced by other structures such as bone against bone when the alar ligament system has failed. Again, caution should be used when extrapolating this data to a clinical scenario. The authors strongly recommend that other findings such as kinesiophobia, anxiety, or muscle guarding also be present when and if a clinician considers a side bending stress test positive for either increased motion or decreased resistance.

Given that the present study was performed on cadaver specimens, it has several limitations. The in vitro conditions of this study are not directly comparable to the conditions present in a clinical scenario. For example, some authors assume that a complete rupture of the alar ligament is always associated with a bone fracture, which is not simulated in this study (Wolfgang et al., 1995). Also, other structures likely to influence upper cervical motion were dissected in order to isolate the alar ligaments' role during side bending (Lenz et al., 2012). Age-related degenerative changes should be taken into consideration in this study (Beyer et al., 2016), since cervical ROM is expected to decrease with age. Upper cervical coupled motion patterns are likely to be influenced by degenerative changes which may also alter facet and ligament orientation. Therefore, the effects of the different tests may be specimen specific. The small sample size should also be considered. Anatomical variations in the cervical spine are numerous (Van Roy et al., 1997) and inter-individual variations in terms of soft tissue stiffness, dysfunction, or morphology (Moore and Dalley, 2006; Osmotherly et al., 2013) are also likely to lead to variations in side bending.

From a clinical point of view, the side bending stress test is intended to identify patients with upper cervical instability. In the presence of a positive test it has been recommended that patients be referred to the appropriate medical professional for further diagnostic testing (Meadows, 1998). In addition, forceful treatment of the upper cervical spine in the presence of upper cervical instability may result in adverse events such as neural damage or vascular injury (Sanchez Martin, 1992; Meadows, 1998; Di Fabio, 1999). The results of this study support the notion that the presence of alar ligament laxity will likely result in increases in motion in both directions of side bending. However, the small amount of motion increase, the fact that the motion increases bilaterally, and the fact that this study was performed in vitro leave some doubt as to the clinical utility of the side bending stress test. Further research in a patient population with suspected upper cervical instability is needed to examine the diagnostic value of the side bending stress test in detecting alar ligament laxity.

5. Conclusion

This in vitro study simulates the clinical application of the side bending stress test with intact and right transected alar ligaments. Both the range of motion and the force of mobilization measured pre and post transection were sensitive to alar ligament transection. Unilateral transection of the alar ligament revealed a predominantly bilateral increase in upper cervical side bending and variability in the mobilization force applied during the test. Additional in vivo studies are needed to validate the results of this study in a clinical setting.

Ethical approval

Research Ethics Committee from UIC-Barcelona. Ref. CBAS-2017-03.

Funding

IAF (Instituto Aragonés de Fomento), through the agreement with the Impact Laboratory of the I3A (Aragón Institute of Engineering Research) of the University of Zaragoza.

Declaration of competing interest

None declared.

Acknowledgement

The authors are thankful to body donors that enable the study of anatomy. Thanks to the Instituto Aragonés de Fomento (IAF), Unidad de Investigación en Fisioterapia at University of Zaragoza, Anatomy Laboratories from Universitat Internacional de Catalunya and the Ethical Committee from UIC-Barcelona for its support. And we also thank to board member of the Basic Science Department from UIC for their help and support.

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Chapter 4

Effect of Alar Ligament Transection on the Rotation Stress Test

The article presented in this chapter shows how alar ligament transection influences the axial rotation range of motion in the upper cervical spine. Right and left axial rotation increased after cutting the right alar ligament: from 33.9 ± 6.6° to 38.5± 9.5° in the right side, and from 28.0± 6.9° to 31.6± 6.5° in the left side. The axial rotation before and after alar ligament transection was compared when the applied load was 0.15 Nm and 0.30 Nm; and with these two loads, the motion was larger after the alar ligament transection. This knowledge about the alar ligaments and the axial rotation is valuable to better understand the clinical application of the rotation stress test.

Hidalgo-García, C., Lorente, A. I., Lucha-López, O., Auría-Apilluelo, J. M., Malo-Urriés, M., Rodríguez-Sanz, J., López-de-Celis, C., Maza-Frechín, M., Krauss, J. and Pérez-Bellmunt, A., 2020. The Effect of Alar Ligament Transection on the Rotation Stress Test: A Cadaveric Study. Clinical Biomechanics, *80, p.105185.*
https://doi.org/10.1016/j.clinbiomech.2020.105185

Clinical Biomechanics 80 (2020) 105185

Contents lists available at ScienceDirect

Clinical Biomechanics

journal homepage: www.elsevier.com/locate/clinbiomech

Lecture

The effect of alar ligament transection on the rotation stress test: A cadaveric study

César Hidalgo-García [a,*], Ana I. Lorente [b,1], Orosia Lucha-López [a], José Manuel Auría-Apilluelo [c], Miguel Malo-Urriés [a], Jacobo Rodríguez-Sanz [a], Carlos López-de-Celis [d,e], Mario Maza-Frechín [b], John Krauss [f], Albert Pérez-Bellmunt [d]

[a] *Unidad de Investigación en Fisioterapia, Universidad de Zaragoza, Spain*
[b] *Impact Laboratory, Aragon Institute of Engineering Research, Universidad de Zaragoza, Spain*
[c] *IDERGO Research Group, Universidad de Zaragoza, Spain*
[d] *Anatomy Unit, Department of Basic Areas, Universitat Internacional de Catalunya, Barcelona, Spain*
[e] *Fundació Institut Universitari per a la recerca a l'Atenció Primària de Salut Jordi Gol i Gurina, Barcelona, Spain*
[f] *School of Health Science, Oakland University, USA*

ARTICLE INFO

Keywords:
Alar ligament
Cervical spine
Upper cervical spine
Physical therapy
Rotation stress test

ABSTRACT

Background: The rotation stress test is a pre-manipulative screening test used to examine upper cervical instability. This in vitro study simulates the clinical application of the rotation stress test before and after alar ligament transection.
Methods: After the dissection of the superficial structures to the alar ligament and the fixation of C2, ten cryopreserved upper cervical columns were manually mobilized in right and left rotation without and with right alar ligament transection. Upper cervical rotation range of motion (RoM) and mobilization torque were recorded using the Vicon motion capture system and a load cell.
Findings: Ligament transection resulted in a larger rotation range of motion in all specimens (contralateral rotation (3.6°, 12.9%) and ipsilateral rotation (4.6°, 13.7%)). The mobilization torque recorded during rotation varied among the different specimens, with a trend towards reduced torque throughout the test in contralateral rotation.
Interpretation: This study simulated the rotation stress test before and after alar ligament transection. Unilateral transection of the alar ligament revealed a bilateral increase of the upper cervical rotation. Additional in vivo studies are necessary to validate the results of this study in patients with suspicion of upper cervical instability.

1. Introduction

The stability of the craniocervical junction is of vital importance because instability at this anatomical site can have life-altering consequences due to damage to neurovascular structures(Tubbs et al., 2012). This stabilization must interact with the extensive motion of the upper cervical spine, especially in axial rotation in the transverse plane(Lummel et al., 2012a).

Although other soft tissue structures including muscles, ligaments, and joint capsules are involved in the stability of upper cervical axial rotation(Brolin and Halldin, 2004), the crucial restraint is the bone-ligament-bone system of the alar ligaments(Crisco et al., 1991a). There are different studies (with anatomical samples or healthy subjects) that have analyzed the influence of the alar ligament in the rotation of the upper cervical spine(Crisco et al., 1991b; Kaale et al., 2008; Panjabi and Dvorak, 1991). If these structures are injured, an increased mobility and diminished resistance in upper cervical axial rotation may be expected.

Pre-manipulative screening is commonly used in clinical practice and has been advocated prior to the treatment of disorders of the upper cervical spine in order to detect potential patients with instability(Kaltenborn, 2012) and to evaluate the integrity of the alar ligaments. One of the described tests for screening upper cervical axial rotation instability is the rotation stress test. In this test, the axis is stabilized by the grasping

* Corresponding author at: Facultad de Ciencias de la Salud de la Universidad de Zaragoza, c/ Domingo Miral s/n 50009, Zaragoza, Spain.
E-mail address: hidalgo@unizar.es (C. Hidalgo-García).
[1] Equal contribution to the first author.

https://doi.org/10.1016/j.clinbiomech.2020.105185
Received 25 September 2019; Accepted 28 September 2020
Available online 1 October 2020

of the lamina with the thumb and index finger of one hand using a lumbrical grip. The head is then rotated to the end of the available range, with the occiput simultaneously moving the atlas with it. According to Osmotherly et al., the range of motion (RoM) during the rotation stress testing in the healthy population should be 21° or less(Osmotherly et al., 2013a). Osmotherly et al. concluded the RoM ranged between 1.7° and 21.5° when comparing neutral and end-range rotation stress test positions during magnetic resonance imaging. This is in contrast with the higher upper cervical rotation (between 20° and 40°) reported in the clinical interpretation of the rotation stress test(Osmotherly et al., 2013a).

The influence of the alar ligament system on the rotation stress test can be examined in vitro in a cadaveric specimen by comparing rotational motion with the alar ligament intact and transected. Panjabi et al. investigated the effect of the transection of an alar ligament in upper cervical rotation of cadaveric specimens(Crisco et al., 1991b). However, Panjabi et al. fixated their specimens through a lower cervical vertebra (C3), and not C2, so their study did not reproduce the same procedure of the rotation stress test. Furthermore, axial rotation was not applied with the same testing parameters as the clinical application of the rotation stress test.

The purpose of this in vitro study was to examine the kinematic behaviour of the upper cervical spine during the rotation stress test with fixation of C2 prior to and following unilateral alar ligament transection.

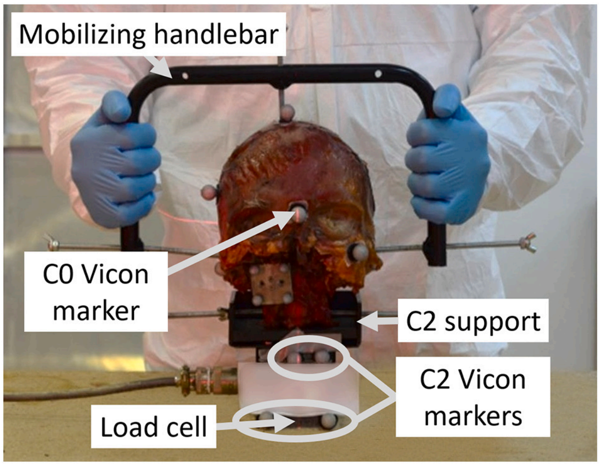

Fig. 1. Specimen set up (with the specimen attached to the load cell, C2 stabilization, Vicon markers and the mobilizing handlebar) prior to upper cervical rotation mobilization.

2. Materials and methods

2.1. Sample

Ten cervical spines and heads from cryopreserved cadavers (9 males, 1 female, mean age: 74 years, range: 63–85 years) were examined in this study. To determine suitability for inclusion, all specimens were visually checked for evidence of prior surgery, trauma, or any anatomical abnormalities that would influence RoM. All specimens were donated to the Universitat Internacional de Catalunya, Spain. The study was approved by the Research Ethics Committee from UIC-Barcelona, Spain. (Ref. CBAS-2017-03).

2.2. Anatomical and biomechanical procedure

All specimens were stored in a freezer at −14 °C and thawed to room temperature 24 h prior to data collection. To prevent dehydration and ensure that specimens remained physiologically viable during this study, the room where the study was conducted was maintained between 17.0° and 17.8° Celsius and the humidity was maintained between 47 and 52%. To prepare each specimen the following procedures were used. First, all spinal segments caudal to C2 were removed by disarticulating C2 from C3 by cutting through the C2-C3 intervertebral disc and zygapophysial facet joint capsules. Second, all muscle tissue in the specimens was removed with care taken not to disrupt any ligamentous tissues. Third, the cranial posterior third of the skull was removed using a wide posterior wedge cut as described by Dvorak et al. to allow for the brain to be removed and the visualization of the foramen magnum(Dvorak et al., 1988; Panjabi and Dvorak, 1991). Integrity of the posterior arch of atlas was maintained during this procedure. Forth, the brainstem, spinal cord, dura and part of the tectorial membrane were carefully removed to expose the alar ligament. Finally, for the purpose of attaching the measurement sensors, the mandible and upper maxilla were removed. Once the specimen was ready for testing, a metallic handlebar was attached to the skull by three points, one through each auditory canal and one through the top of the head. The handlebar is pictured on Fig. 1, and was designed to move the head without contacting any sensors attached to the specimens.

After the specimens were prepared they were then fixed to the load cell (capacity of 56 Nm and stiffness of 2.2×10^4 Nm/rad for rotation, MC3–6-100, Advanced Mechanical Technology Inc., Watertown, MA, USA) which measured the torque required to generate axial rotation of the head. The amount of force applied was calculated from the torque measured by the load cell, using a distance of 150 mm which was the distance between the handlebar and an estimated axis of rotation symmetrically located on the specimen. Specifically, the load cell was attached to a metalic support which was attached by a screw to the C2 vertebra. C2 was attached in the mid position without any rotation. The head was aligned with this position before each axial rotation motion was performed. To find the head neutral position, a symmetrical vertical line was marked on the center of the face, and an anatomical Frankfurt horizontal plane was laterally marked on the head (a line through the upper margin of the external auditory meatus and the infraorbital foraminae). These two lines were aligned with the horizontal and vertical references given by two red light lasers calibrated to be horizontal and vertical. Neutral head position was checked before C2 fixation to ensure that both were in a neutral position after C2 fixation.

A Vicon motion capture system (TS series, Vicon, Oxford, UK), consisting of four 1.000 Hz cameras, was used to examine the 3D motion of the head over C2 during axial rotation. The mean error exhibited after the system calibration was 0.034 mm (0.0130° for angular measurements). Retroreflective spherical markers placed on the head and C2 (Fig. 1) were used to define head and C2 local coordinate systems. Vicon systems have demonstrated good motion acquisition accuracy in previous studies and are used in a wide variety of biomechanical analysis (Windolf et al., 2008), including studies examining the cervical spine (Yoganandan et al., 2007). Obtaining anatomical coordinate systems from Vicon markers is considered a valid and reliable measurement practice(Cappozzo et al., 2005; Wu et al., 2002). The implementation of single markers or marker clusters used in this study, and the attachment of the marker to the plate rather than directly to C2, because of C2's limited surface area, is also considered a valid and reliable measurement practice(Lessley et al., 2011) (Fig. 1).

Each specimen was rotated five times. The first two axial rotation motions were used as warm-up motions to reduce the influence of soft tissue viscoelasticity(Bernhardt et al., 1999), with all measurements being recorded on the third (alar ligament intact) and fifth (right alar ligament cut) motions. For the purpose of simulating the passive rotation stress test, all movements were induced manually in the transverse plane until a marked resistance was perceived by the tester. All axial rotation movements for all the specimens were performed by the same researcher with more than 15 years of experience in manual therapy and applying the rotation stress test. After the experimentation, the degree of motion and torque was measured at the end-range of the simulated rotation stress test without and with right alar ligament dissection. In addition, range of motion in both conditions was measured with standardized 1 N (0.15 Nm), 2 N (0.30 Nm), and 3 N (0.45 Nm) forces of mobilization.

2.3. Statistical analysis

SPSS statistical software (version 20.0) for Windows was used for all statistical analyses. A descriptive analysis of the axial rotation angles and torques applied during the experiment was performed. The differences between normal and transected specimens was compared using the Wilcoxon rank test. The Shapiro-Wilk test was used to identify the normal distribution of the sample. The level of significance was set at alpha $\leq$0.05.

3. Results

Fig. 2 illustrates the amount of torque applied and the resultant axial rotation movement for all ten specimens with both alar ligaments intact (illustrated in black) and with the right alar ligament cut (illustrated in grey). When reading the graphs, positive values indicate right axial rotation, and negative values indicate left axial rotation. Table 1 contains the axial rotation angles recorded for each specimen when the applied torques were 0.15 Nm, 0.30 Nm and 0.45 Nm, as well as the torque applied to achieve maximum RoM. Table 2 shows the relationship between the amount of upper cervical right and left rotation and the associated mobilization torque at a standardized 0.15 Nm, 0.30 Nm, 0.45 Nm and at maximal torque with an intact and then transected right

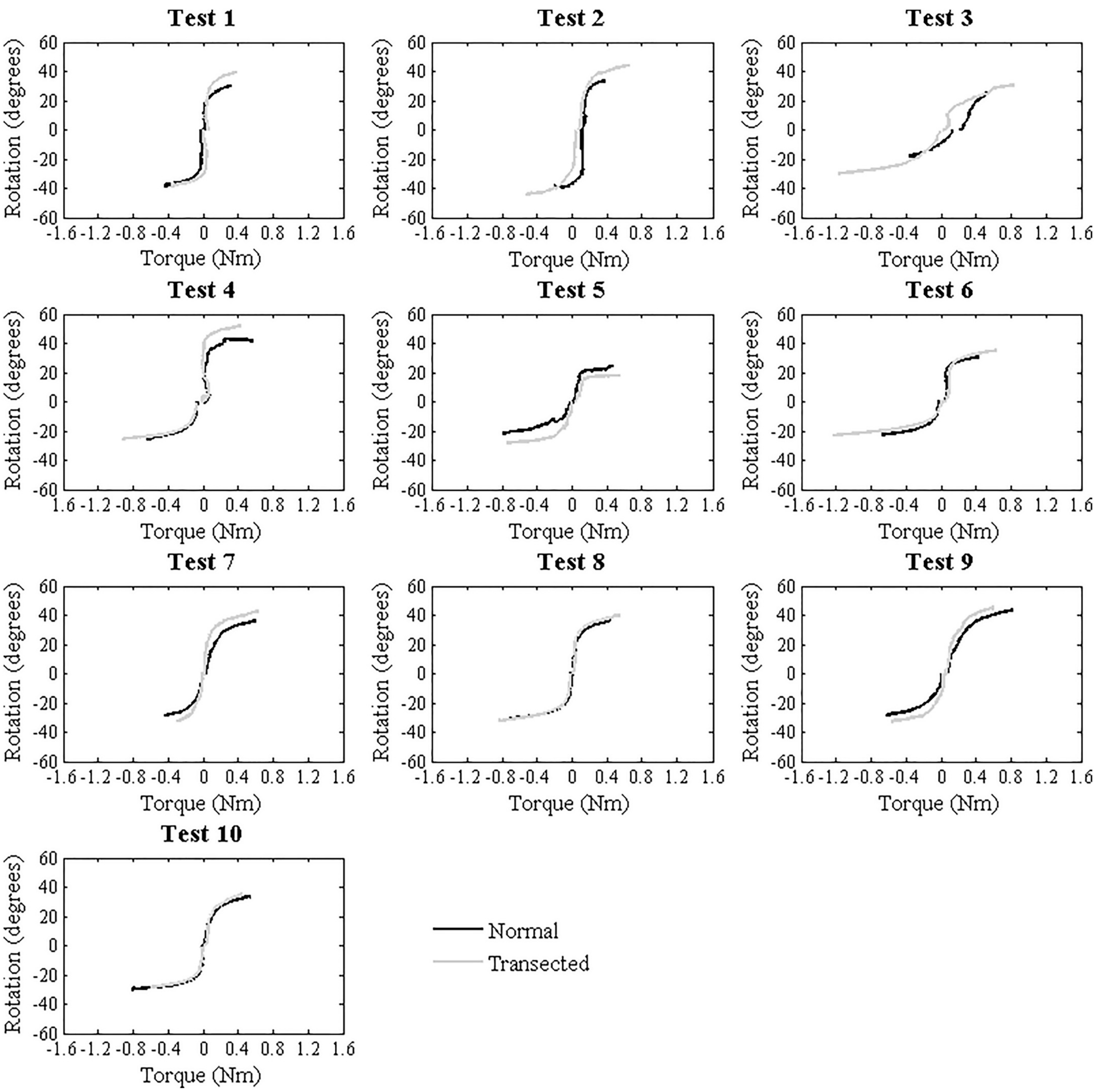

Fig. 2. Rotation of the C0-C2 segment and mobilization force before and after the right alar ligament transection. Negative values represent right rotation and positive values represent left rotation (Black line: intact specimen, Grey line: transected specimen).

Table 1
Mobilization torque and RoM of the upper cervical spine in right and left rotation (°) at standardized 0.15 Nm, 0.30 Nm, 0.45 Nm torques (1 N, 2 N and 3 N forces) and at the end-range (torque max, RoM max) in the 10 specimens with intact (normal) and transected (right alar ligament dissected) conditions.

Test		Torque (Nm)									
		Right					Left				
		0.15 Nm	0.30 Nm	0.45 Nm	T Max	RoM Max	0.15 Nm	0.30 Nm	0.45 Nm	T Max	RoM Max
1	Normal	26.1	30.0		0.32	30.8	33.6	36.0		0.43	38.0
	Transected	33.9	38.1		0.37	39.4	35.2	37.1		0.34	41.1
	Difference	*7.8*	*8.1*		*0.05*	*8.6*	*1.6*	*1.1*		*−0.09*	*3.1*
2	Normal	5.7	32.6		0.37	33.5	38.5			0.19	39.0
	Transected	26.3	38.7	41.3	0.64	44.6	37.8	41.8	43.1	0.52	43.8
	Difference	*20.6*	*6.1*		*0.27*	*11.1*	*−0.7*			*0.33*	*4.8*
3	Normal	0.0	7.5	21.8	0.52	24.8	13.6	16.4		0.37	17.6
	Transected	15.7	20.2	23.7	0.84	30.7	13.2	19.6	23.2	1.17	29.6
	Difference	*15.7*	*12.7*	*1.85*	*0.32*	*5.9*	*−0.4*	*3.2*		*0.80*	*12.0*
4	Normal	38.0	42.7	42.5	0.57	43.9	15.6	20.7	22.7	0.64	25.4
	Transected	47.9	50.3		0.42	52.6	13.4	19.7	21.8	0.93	25.4
	Difference	*9.9*	*7.6*		*−0.15*	*8.7*	*−2.2*	*−1.0*	*−0.9*	*0.29*	*0.05*
5	Normal	21.3	22.1	24.2	0.46	24.6	12.2	14.5	17.8	0.79	21.1
	Transected	16.0	17.5	17.9	0.54	18.0	20.2	24.5	26.3	0.75	28.0
	Difference	*−5.3*	*−4.6*	*−6.3*	*0.08*	*−6.6*	*8.0*	*10.0*	*8.5*	*−0.04*	*6.9*
6	Normal	26.7	29.2		0.42	30.5	15.1	18.8	20.4	0.69	22.1
	Transected	27.2	31.3	33.4	0.63	35.3	12.6	15.5	17.3	1.24	22.7
	Difference	*0.5*	*2.1*		*0.21*	*4.9*	*−2.5*	*−3.3*	*−3.1*	*0.55*	*0.6*
7	Normal	22.7	31.0	34.1	0.60	36.9	21.5	26.3		0.43	28.4
	Normal	33.0	37.6	39.7	0.61	42.8	27.4	32.5		0.31	32.8
	Difference	*10.3*	*6.6*	*5.6*	*0.01*	*5.9*	*5.9*	*6.2*		*−0.12*	*4.4*
8	Normal	30.0	34.3		0.43	36.5	23.2	26.5	28.4	0.72	30.8
	Transected	32.4	36.5	38.6	0.54	40.4	22.6	26.6	28.5	0.84	32.1
	Difference	*2.4*	*2.2*		*0.11*	*3.9*	*−0.6*	*0.1*	*0.1*	*0.12*	*1.3*
9	Normal	16.1	31.1	37.6	0.82	43.5	18.9	23.7	26.0	0.63	28.0
	Transected	25.7	37.6	42.2	0.60	45.5	25.7	29.3	31.2	0.57	32.3
	Difference	*9.6*	*6.5*	*4.6*	*−0.22*	*2.0*	*6.8*	*5.6*	*5.2*	*−0.06*	*4.3*
10	Normal	24.3	30.1	32.5	0.52	33.7	23.4	26.0	27.3	0.82	29.5
	Transected	26.4	31.9		0.43	35.6	22.4	25.2	26.7	0.60	28.1
	Difference	*2.1*	*1.8*		*−0.09*	*1.9*	*−1.0*	*−0.8*	*−0.6*	*−0.22*	*−1.4*

Table 2
Mean, standard deviation (SD) and confidence interval (CI 95%) of all the specimens ($n = 10$) for right and left rotation in normal condition and after right alar ligament transection.

Right rotation					
		0.15 Nm	0.30 Nm	T Max	RoM Max
Normal	Mean (SD)	21.1 (11.3)	29.1 (9.1)	0.6 (0.1)	33.9 (6.6)
	CI	13.0/29.1	22.5/35.6	0.4/0.6	29.1/38.6
Transected	Mean (SD)	28.4 (9.3)	34 (9.5)	0.6 (0.1)	38.5 (9.5)
	CI	21.8/35.1	27.2/40.8	0.5/0.7	31.7/45.3
P-value		**0.017***	**0.017***	0.333	**0.037***
Left rotation					
		0.15 Nm	0.30 Nm	T Max	RoM Max
Normal	Mean (SD)	21.6 (8.6)	23.2 (6.5)	0.6 (0.2)	28 (6.9)
	CI	15.4/27.7	18.2/28.2	0.4/0.7	23.1/32.9
Transected	Mean (SD)	23.1 (8.8)	27.2 (8.2)	0.7 (0.3)	31.6 (6.5)
	CI	16.8/29.4	21.3/33.1	0.5/1.0	26.9/36.3
P-value		0.646	0.173	0.285	**0.017***

* Statistical significance $p \leq 0.05$.

alar ligament for the whole sample.

During right axial rotation, the average range with intact alar ligaments was 33.9° (SD 6.6). All specimens demonstrate an increase in axial rotation angles with the transection of the right alar ligament (average increase 4.6°, $p = 0.037$) except for specimen 5 (4.6°, SD 4.9° average increase in the end-range). This is illustrated in Fig. 2, where the grey line is higher or equal to the black one for a given torque value, except for specimen 5 in right rotation. An increased RoM in right rotation was observed when comparing the tests with and without transection at standardized torques of 0.15 Nm (7.3°) and 0.30 Nm (4.9°).

During left axial rotation, the average range with intact alar ligaments was 28° (SD 6.9). All specimens demonstrate an increase in axial rotation angles following the transection of the right alar ligament (average increase 3.6°, $p = 0.017$) except for specimen 10. Specimen 10 showed a reduction of −1.4° although the torque used to achieve end-range was 0.22 Nm lower after the alar ligament was cut as compared to when it was intact. The increased RoM in left rotation was observed when comparing the tests without and with transection at standardized torques of 0.15 Nm (1.5°), 0.30 Nm (2.3°), and 0.45 Nm (3.8°). This phenomenon is not the same throughout the specimens: specimens 1, 5, 7 and 9 had an increase on the angles for the same torque examined when comparing with the normal specimens, but specimens 3, 6 and 8 showed a decreased angle after the transection with the inferior torque value application but not with higher torque values (end-range).

4. Discussion

This study simulated the clinical application of the rotation stress test before and after transection of the alar ligament. The results of this study show that unilateral transection of the alar ligament resulted in a bilateral increase of the upper cervical axial rotation RoM, and a variable stiffness encountered throughout the test.

4.1. Range of motion during the rotation stress test

The upper cervical axial rotation ranges measured in this study are within the lower and higher clinical bound estimations of movement that have been suggested previously in the literature for this test (Osmotherly et al., 2013a). Conversely, the axial rotation motion from our in vitro study is substantially larger than the cut-off value of in vivo rotation of 21° or less that has been proposed in normal subjects by Osmotherly et al.(Osmotherly et al., 2013a). High variability of rotational mobility at the craniocervical junction has been reported(Lummel et al., 2012b) and is present in our sample. Differences between this in vitro study and Osmotherly et al. could be due to the resection of all superficial tissues, which likely provide some restriction to axial rotation. In addition, in vitro examination allows C2 to be fixated, creating a stable axis of motion during rotation, and a consistent zero position for measurement that is unlikely during in vivo examination. Although variability in the RoM is frequent in the upper cervical spine, 21° or less may be considered a conservative cut-off value for a normal rotation stress test.

Tisherman et al. (2020) reported 73.5° of rotation in the transverse plane with an increase of 4.1% after unilateral transection of the alar ligament(Tisherman et al., 2020). Our sample presented 61.9° in the transverse plane with an increase of 13.2% after unilateral alar ligament transection. Dvorak et al. obtained an increase of 10.5° (29% of the initial 36° range of motion) only in the contralateral rotation and not in ipsilateral rotation after the unilateral dissection of an alar ligament (Dvorak et al., 1988). However, Panjabi et al. obtained a significant increase in both rotations (8.1% of the initial 40.7° ipsilateral range of motion and a 10.5% of the initial 40° range of contralateral rotation) (Crisco et al., 1991b). In our study, the total increase in upper cervical ipsilateral rotation and contralateral rotation after unilateral dissection of an alar ligament was 4.6° (13.7% of the initial 33.9° range of motion) and 3.6° (12.9% of the initial 28° range of motion) respectively. Although the methodology of these studies do not allow direct comparison due to the differences in vertebral fixation and the mobilization torque, our results found an increase in both rotations after a unilateral alar ligament transection.

4.2. Stiffness during the rotation stress test

Findings from this study indicate that stiffness encountered during the rotation stress test changes during right and left rotation following the transection of the right alar ligament. When the mobilization torque is standardized, most of the specimens showed an increase in the rotation RoM at the same mobilization torque. However, this was not present in all specimens. Anatomical variations in the upper cervical spine including the specific orientation of the alar ligaments are frequent and could be an explanation for differing results in a specific specimen (Osmotherly et al., 2013b). The increase in the mobilization torque at the end-range found in some dissected specimens could be produced by other sources including bone contacting bone once the alar ligament system has failed.

This finding supports the notion that reduced resistance during the rotation stress test seems to be a sensitive parameter for instability (Panjabi et al., 1991). Kaale et al. concluded that it is possible to detect joint dysfunction in the upper cervical spine during clinical examination based on an assessment of the quality of occipito-atlanto-axial rotation performed by one examiner and compared to MRI in a chronic whiplash sample(Kaale et al., 2008).

There are several limitations to this study. These include a small sample size, the in vitro design of the study, and the inability to simulate the dynamic responses of a patient present during the clinical application of the rotation stress test. Numerous structures were removed in the preparation of the specimens used in this study including bone, ligament and myofascial tissues that may influence movement of the upper cervical spine(Lenz et al., 2012). Some authors assume that a complete rupture of the alar ligament is always associated with a bone fracture which is not simulated in this study(Willauschus et al., 1995). In addition, RoM is expected to decrease with age(Beyer et al., 2016). Our predominant male sample (9/10) may limit the generalizability of these results to a female population. Age, joint orientation, articular surface area, and ligament orientation may also influence motion coupling patterns. Anatomical variations in the cervical spine are numerous and inter-individual variations in terms of soft tissue stiffness or morphology may also contribute to variations in the results. Therefore, the degree of rotation before and following the transection of the alar ligament may be specimen specific(Cattrysse et al., 2011).

From a clinical point of view, the rotation stress test is used to identify patients with upper cervical instability. If upper cervical instability can be identified during the physical examination, then it is possible to select interventions that minimize the risk of damage to the local neural and vascular structures(Di Fabio, 1999; Meadows, 1998; Rushton et al., 2014). This study explores the effects of alar ligament transection on the rotation stress test using an in vitro procedure. The fact that the increase of rotation RoM found in this study was not present in all specimens, and that there was high variability in the rotational motion present in the upper cervical spine, challenges the notion that the rotation stress test would have detected ligament damage in all our specimens. Also, it is unknown if the practitioner would be able to detect a positive or negative finding during the rotation stress test in a clinical setting. Additional studies are recommended to validate the rotation stress test in a patient population with confirmed upper cervical instability.

5. Conclusion

This study has examined the application of the rotation stress test in vitro before and after alar ligament transection. Both the range and quality of motion were impacted by alar ligament transection. Unilateral transection of the alar ligament caused a bilateral increase in upper cervical rotation and a trend towards a decrease in the amount of torque required throughout the test. Additional in vivo studies are necessary to validate the results of this study in a clinical population with suspected upper cervical instability.

Funding

This work was supported by the IAF (Instituto Aragonés de Fomento), Spain.

Declaration of Competing Interest

All authors have approved the final version of the manuscript and agree to the transfer of copyright to your Journal. There are no relationships, financial or otherwise, that wuld create any conflicts of interest.

Acknowledgement

The authors are thankful to the body donors that enabled this study of anatomy. Thanks to the Instituto Aragonés de Fomento (IAF, through the agreement with the Impact Laboratory of the Aragón Institute of Engineering Research, I3A), Unidad de Investigación en Fisioterapia at University of Zaragoza, Anatomy Laboratories from Universitat Internacional de Catalunya and the Ethical Committee from UIC-Barcelona for its support. We also thank board members of the Basic Science Department from the UIC for their help and support.

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Chapter 5

Intersegmental Kinematics of the Upper Cervical Spine

The article presented in this chapter describes how alar ligament transection influences the intersegmental kinematics and stiffness at the craniovertebral junction. The motion in the three planes was evaluated: flexion and extension, bilateral lateral bending, and bilateral axial rotation. After unilateral alar ligament transection, C0-C2 range of motion increased in the three planes of movement, while intersegmental alterations were not always observed.

The text presented in this chapter is the one published in the journal *Spine*. The final published article is available in the journal's website:

*Lorente, A.I., Hidalgo-García, C., Rodríguez-Sanz, J., Maza-Frechín, M., Lopez-de-Celis, C. and Pérez-Bellmunt, A., 2021. Intersegmental Kinematics of the Upper Cervical Spine: Normal Range of Motion and its Alteration After Alar Ligament Transection.*Spine *, 46 (24), p.E1320.*

https://doi.org/10.1097/BRS.0000000000004167

Spine

SPINE Volume 46, Number 24, pp E1320–E1326

BIOMECHANICS

Intersegmental Kinematics of the Upper Cervical Spine

Normal Range of Motion and Its Alteration After Alar Ligament Transection

Ana I. Lorente, MSc,[a] César Hidalgo-García, PhD,[b] Jacobo Rodríguez-Sanz, PhD,[c,d]
Mario Maza-Frechín, PhD,[a] Carlos López-de-Celis, PhD,[c,d,e] and Albert Pérez-Bellmunt, PhD[c,d]

Full text:

https://doi.org/10.1097/BRS.0000000000004167

Chapter 6

Kinematics after Occipital-Atlas Stabilization

The article presented in this chapter shows how occipital-atlas stabilization influences the upper cervical spine kinematics. The range of motion in the three anatomical planes was considered (flexion and extension, lateral bending, and axial rotation), comparing it before and after occipital-atlas stabilization. In the three planes, the C0-C2 range of motion decreased with the stabilization of C0-C1. The greatest reduction was in the frontal plane, where the lateral bending was reduced by the half (55.3%). A reduction close to this one was also observed in the sagittal plane (flexion-extension), where the motion was reduced by 46.9%. Lastly, the lowest reduction was in the transverse plane, with a reduction of 15.6% in the axial rotation.

Hidalgo-García, C., Lorente, A.I., López-de-Celis, C., Lucha-López, O., Malo-Urriés, M., Rodríguez-Sanz, J., Maza-Frechín, M., Tricás-Moreno, J.M., Krauss, J. and Pérez-Bellmunt, A., 2021. Effects of Occipital-atlas Stabilization in the Upper Cervical Spine Kinematics: an in Vitro Study. Scientific Reports *, 11(1), p.1-13.*
https://doi.org/10.1038/s41598-021-90052-6

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Effects of occipital-atlas stabilization in the upper cervical spine kinematics: an in vitro study

César Hidalgo-García[1,5]✉, Ana I. Lorente[2,5], Carlos López-de-Celis[3], Orosia Lucha-López[1], Miguel Malo-Urriés[1], Jacobo Rodríguez-Sanz[3], Mario Maza-Frechín[2], José Miguel Tricás-Moreno[1], John Krauss[4] & Albert Pérez-Bellmunt[3]

This study compares upper cervical spine range of motion (ROM) in the three cardinal planes before and after occiput-atlas (C0–C1) stabilization. After the dissection of the superficial structures to the alar ligament and the fixation of C2, ten cryopreserved upper cervical columns were manually mobilized in the three cardinal planes of movement without and with a screw stabilization of C0–C1. Upper cervical ROM and mobilization force were measured using the Vicon motion capture system and a load cell respectively. The ROM without C0–C1 stabilization was 19.8° ± 5.2° in flexion and 14.3° ± 7.7° in extension. With stabilization, the ROM was 11.5° ± 4.3° and 6.6° ± 3.5°, respectively. The ROM without C0–C1 stabilization was 4.7° ± 2.3° in right lateral flexion and 5.6° ± 3.2° in left lateral flexion. With stabilization, the ROM was 2.3° ± 1.4° and 2.3° ± 1.2°, respectively. The ROM without C0–C1 stabilization was 33.9° ± 6.7° in right rotation and 28.0° ± 6.9° in left rotation. With stabilization, the ROM was 28.5° ± 7.0° and 23.7° ± 8.5° respectively. Stabilization of C0–C1 reduced the upper cervical ROM by 46.9% in the sagittal plane, 55.3% in the frontal plane, and 15.6% in the transverse plane. Also, the resistance to movement during upper cervical mobilization increased following C0–C1 stabilization.

The occipital-atlas (C0–C1) and atlas-axis (C1–C2) segments join the head to the most mobile region of the spine. The lack of intervertebral discs, the horizontal nature of the joints, and the specialized muscles and ligaments of these segments produce complex three-dimensional kinematics[1]. Due to the complex kinematics within the upper cervical spine, it has been suggested that it be considered as one functional unit, especially in axial rotation. Bogduk and Mercer (2000) proposed that C0–C1 moves primarily in flexion–extension, while C1–C2 mainly rotates. However, Bogduk and Mercer's findings suggest that interactions between C0–C1 and C1–C2 vary depending on the specific planes of movement[2].

In the sagittal plane, most studies agree that the average motion for C0–C1 and C1–C2 is 14–15° and 10–21°, respectively[2,3]. However, within the literature, there seems to be variability in the specific contributions of C0–C1 versus C1–C2 during sagittal plane movements. Chancey et al. (2007) described that 41–45% of the upper cervical flexion and 69–71% of the extension occurred in C0–C1[4], Fujimori et al. (2013) concluded that C0–C1 works mainly for flexion-extension[5] and Bogduk and Mercer (2000) stated that C0–C1 facilitates C1–C2 motion and that C1–C2 is moved passively by forces coming from C0–C1[2].

Upper cervical spine range of motion (ROM) in the frontal plane is very limited[6]. Bogduk and Mercer (2000) concluded that C0-C2 move and function as one unit[2]. Limitations in side bending ROM at C1–C2 is thought to be caused by contralateral alar ligament tension or by the impaction of the lateral mass of atlas on the odontoid process[7]. Osmotherly et al. (2012) concluded that any lateral flexion movement of the upper cervical spine (UCS), when C2 is stabilized, is a sign of craniocervical instability[8].

Approximately 60% of the total cervical ROM in the transverse plane is produced by the UCS[9]. Salem et al. (2013) indicated that C1–C2 shows the largest magnitude of axial rotation with a minimal contribution from C0–C1[10]. Kang et al. (2019) commented that axial ROM at C0–C1 has rarely been examined in cadaver

[1]Unidad de Investigación en Fisioterapia, Facultad de Ciencias de la Salud de la Universidad de Zaragoza, c/ Domingo Miral s/n, 50009 Zaragoza, Spain. [2]Impact Laboratory, Aragon Institute of Engineering Research, Universidad de Zaragoza, Alcañiz, Spain. [3]ACTIUM Functional Anatomy Group, Universitat Internacional de Catalunya, Barcelona, Spain. [4]School of Health Sciences, Oakland University, Rochester, MI, USA. [5]These authors contributed equally: César Hidalgo-García and Ana I. Lorente. ✉email: hidalgo@unizar.es

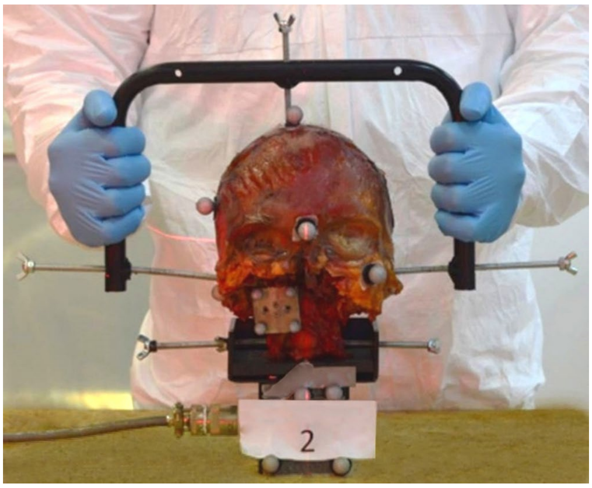

Figure 1. C0-C2 specimen: starting set up of the test.

studies[9]. In fact, several authors have disregarded C0–C1 motion completely when studying upper cervical axial rotation[11–14].

However small the actual motion occurring during rotation at C0–C1, there is an emerging body of evidence supporting the notion that C0–C1 plays a relevant role in the rotation ROM at C1–C2. Improvement of C1–C2 rotation has been demonstrated following the application of manual therapy in the form of translatoric mobilization to C0–C1 in participants with restricted UCS axial rotation[15], patients with cervicogenic headache[16], and patients experiencing chronic cervicalgia[17–19]. This approach is based on a rationale that restricted mobility of the C0–C1 segment could limit C1–C2 movement during rotation due to the alar ligament connection across each joint[20,21]. The purpose of this study is to compare upper cervical ROM in the three cardinal planes before and after C0–C1 stabilization using an in vitro design.

Methods

Sample. Ten cervical spines and heads from cryopreserved cadavers (9 males, 1 female, mean age: 74 years, range 63–85 years) were examined. All specimens were visually checked for any anatomical condition that would influence ROM. In addition, all samples were required to be free of any disease or contamination. All specimens were donated to Universitat Internacional de Catalunya. Informed consent was obtained from a next of kin and/or legal guardian of the cadaver. The study was approved by a Research Ethics Committee from UIC-Barcelona (Ref. CBAS-2017-03) and all methods were carried out in accordance with relevant guidelines and regulations.

Anatomical and biomechanical procedure. This study examines the kinematic behavior of the upper cervical spine during movements of the head in the three cardinal planes before and following a screw stabilization at C0–C1.

All specimens were stored at – 14 °C and thawed to room temperature 24 h before testing. The preparation procedure was as follows: First, all spinal segments caudal to C2 vertebra were removed by disarticulating C2–C3 by cutting through the intervertebral disc and zygapophysial facet joint capsules. Second, all muscle tissue was removed without disrupting ligamentous tissues. Third, the cranial posterior third of the skull was removed[22] to extract the brain and visualize the foramen magnum. The integrity of the posterior arch of atlas was maintained. Forth, the brainstem, spinal cord, dura, and part of the tectorial membrane were removed to expose the alar ligament. Finally, to allow the attachment of the measurement sensors, the mandible, and upper maxilla were removed. Afterward, a metallic handlebar was attached to the skull by three points: one in each auditory canal and one at the top of the head (Fig. 1). The handlebar was designed to move the head without contacting any attached sensors.

The C2 vertebra was then fixed to the load cell (MC3-6-100 Force and Torque Sensor, Advanced Mechanical Technology Inc., Watertown, USA), which measured the torque required to generate the movement in the three cardinal planes. The C2 vertebra was screwed to a metallic support, which was attached to the load cell. The specimen was kept in an upright position (head on top and C2 below) and the three anatomical planes were aligned with the three axes of the load cell. The tester moved the skull from the posterior part of the specimen (Fig. 1).

The force applied by the tester when moving the specimens was converted to newtons from the torque measured by the load cell. The distances between the handlebar and the estimated axes of rotation were used for this calculation: 130 mm for flexion–extension and lateral flexion movements (the height between the center of the hands and the mid-height of C2, as both hands were at the same level), and 150 mm for rotation (the half of the metallic handlebar width). These two measurements were approximated to those two values due to the small

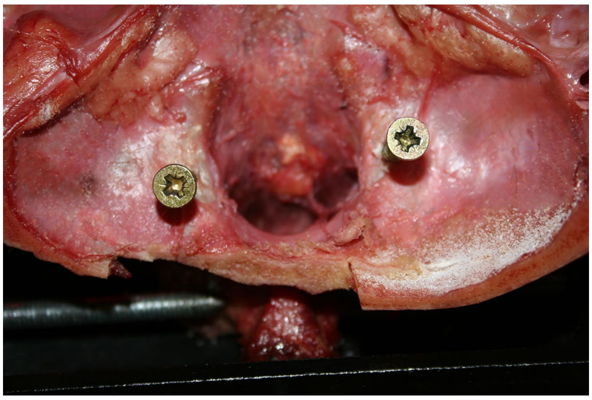

Figure 2. Screw stabilization of occipital-atlas (C0–C1) segment.

variations shown by the instantaneous centres of rotation within individual segments[2,23]. Therefore, the values reported in newtons represent the total load from both hands of the tester in the main plane of the motion.

C2 and the head were aligned in the mid position before each test. To find the mid position, a Frankfurt horizontal plane, which can be considered the physiologic horizontal reference[24], was laterally marked on the head (through the external auditory meati and the infraorbital foraminae), and a vertical line was marked on the center of the face. This vertical line was a straight up mark from the centre of the chin to the centre of the forehead, running through the centre of the nose. These two markings were aligned with references given by two red light lasers calibrated to the horizontal and vertical.

An optical motion capture system (TS Series, Vicon, Oxford, UK) of four cameras tracked the motion of the head, C1, and C2. The measurement error of this system is 0.0130°, therefore the motions are described with one significant digit. Retroreflective spherical markers were directly placed on the head with glue (Loctite Super Glue-3, Henkel, Germany) (Fig. 1). For C1 a total of four markers were attached on a metallic plate, which was screwed to the vertebra (two parker screws of 8 mm). The plate and its markers were positioned so that there was no interference with the motion between C1 and the skull or C2. The C1 motion was tracked to assess the C0–C1 motion pre- and post-screw fixation. Finally, for C2, markers were fixed on the load cell attached to the C2 vertebra.

To calculate the local coordinate systems of the head, C1, and C2, a 3D measuring device (FaroArm, FARO Technologies, Lake Mary, FL, USA) was installed near the load cell, and anatomical landmarks were measured on the (1) skull: right and left auditory meati and right infraorbital foraminae, (2) C1: symmetrical right and left landmarks on the transverse processes, anterior and posterior tubercles, and (3) C2: symmetrical right and left landmarks on the transverse processes, lowest anterior central point on the body, and lowest central point on the spinous process. Using these landmarks for each segment, the coordinate systems had the X-axis pointing forward, the Y-axis pointing from left to right, and completing a right-hand-oriented coordinate system, the Z-axis pointed downwards. By using both the Vicon system and FaroArm it was possible to measure the motion of each segment. The equations required to define the local coordinate systems can be found in Slykhouse et al. (2019)[25]. The coordinate transformation between FaroArm, the optical markers, and the bones has been previously described in detail by Shaw et al. (2009)[26].

Synchronized data collection from both the load cell and motion capture systems, was made possible by the installation of a manual trigger that started both systems simultaneously. Both records ended after a pre-defined time of 15 or 20 s, depending on the movement. To compare the motion among all the specimens, the motion was measured at four different instances with the same load: 1 N, 2 N, 3 N and 4 N. Additionally, the maximum load applied and the maximum range of motion was also analyzed.

Specimens were moved in each plane four times, and always in the same order starting from the neutral position: flexion–extension, right-left lateral flexion, and right-left axial rotation. The first two motions were used as a warm-up to reduce the influence of soft tissue viscoelasticity[27]. Measurements were recorded on the third (prior to C0–C1 stabilization) and forth (post C0–C1 stabilization). All pre-C0–C1 stabilization movements were performed first; then, the post-C0–C1 stabilization movements were performed using the order indicated at the beginning of the paragraph. For the C0–C1 screw stabilization, the occipital entry point of the screw was approximately 5 mm lateral to the foramen magnum, pointing in the direction of and penetrating into the lateral mass of atlas (Fig. 2). The adequacy of screw placement was monitored visually and the C0–C1 and C1–C2 mobility was checked after the screw placement. Approximately 10 mm of the unthreaded portion of the screw remained protruded following screw fixation. All movements were induced manually until a marked resistance was perceived by the tester, a researcher with more than 15 years of clinical experience treating patients with upper cervical impairments who was also a credentialed manual therapist. To prevent dehydration and ensure physiological viability, the dissection room was maintained with a temperature between 17.0° and 17.8° Celsius, and a humidity between 47 and 52%.

Movement	Normal (degrees) C0–C1	Normal (degrees) C1–C2	C0C1 stab (degrees) C0–C1	C0C1 stab (degrees) C1–C2	C0–C1 Movement restriction with C0–C1 stab
Flexion	− 5.8 to 15.2	2.8–23.5	− 0.9 to 6.2	3.7–15.5	74.4%
Extension	2.5–20.8	− 0.4 to 8	0.5–9	0.7–10.2	
Lateral flex.—right	0.9–8.8	0.4–4	− 0.1 to 1.2	− 0.2 to 4.2	76.9%
Lateral flex.—left	1.7–6.7	0.2–5.2	0.3–2.6	0.2–4.1	
Rotation—right	− 2.1 to 6.7	21.9–42.9	− 5.8 to 5.7	19.4–42.1	90.9%
Rotation—left	− 0.9 to 10.4	13.5–37.6	− 3.4 to 3.4	13.1–35.2	

Table 1. Minimum and maximum intervertebral motion (in degrees) for C0–C1 and C1–C2 in the cardinal planes in both conditions: normal and C0–C1 stabilization (C0–C1 stab). These are the values for two specimens in each condition, and the rest of the specimens showed values between this range. The percentages show the C0–C1 movement restriction with C0–C1 stabilization.

Statistical analysis was conducted using SPSS 23.0 (IBM, Armonk, New York). The mean and standard deviation were calculated for each variable. A Wilcoxon Signed Rank Test was performed to analyze intergroup differences, with a significance level set at $p < 0.05$.

Ethical approval. Research Ethics Committee from UIC-Barcelona. Ref. CBAS-2017-03.

Results

Table 1 shows the minimum and maximum segmental motion measured for C0–C1 and C1–C2 in the cardinal planes without and with C0–C1 stabilization. Following stabilization, movement between occipital and atlas was reduced by 74.6%, 76.8%, and 90.9% in the sagittal, frontal, and transverse planes, respectively.

Upper cervical sagittal plane mobility. Figure 3 illustrates the amount of force applied and the resultant flexion and extension movement for all ten specimens without (illustrated in black) and with C0–C1 stabilization (illustrated in grey). Positive values indicate extension, and negative values indicate flexion. Table 2 contains the angles recorded for each specimen when the applied forces were 1 N, 2 N, 3 N, and 4 N, as well as the force applied to achieve maximum ROM with non-stabilized and stabilized C0–C1 configurations.

During upper cervical flexion, the end ROM without C0–C1 stabilization was 19.8° ± 5.2°, with an average maximum force of 5.6 N ± 1.5 N. All specimens demonstrate a reduction in flexion after C0–C1 stabilization (averaged end ROM of 11.5° ± 4.3°). The average maximum force was 7.2 N ± 5.3 N. Following C0–C1 stabilization, flexion ROM decreased during all standardized forces.

During upper cervical extension, the end ROM without C0–C1 stabilization was 14.3° ± 7.7°, with an average maximum force of 6.8 N ± 2.6 N. All specimens demonstrated a reduction in extension ROM following the stabilization of C0–C1 (6.6° ± 3.5° with an average maximum force of 9.8 N ± 4.4 N) during all standardized forces except specimen 7. However, if flexion and extension ROM are grouped together, specimen 7 demonstrated a reduction in ROM of 17.0° following C0–C1 stabilization.

Upper cervical frontal plane mobility. Figures 4 and 5 represent the force applied and the resultant movement for lateral flexion and axial rotation, respectively. In Fig. 4, positive values indicate right lateral flexion, and negative values mean left lateral flexion. In Fig. 5, positive values indicate right axial rotation, and negative values indicate left axial rotation. Tables 3 and 4 contain the ROM for lateral flexion and rotation at 1 N, 2 N, 3 N, and 4 N, as well as the force applied to achieve maximum ROM (without and with C0–C1 stabilization). The ROM values from flexion to the zero position have not been included (empty boxes) in the table.

During upper cervical right lateral flexion, the end ROM without C0–C1 stabilization was 4.7° ± 2.3°, with an average maximum force of 6.5 N ± 1.9 N. Following C0–C1 stabilization, all specimens demonstrated a reduction in ROM (2.3° ± 1.4°) at all standardized forces with an average maximum force of 7.6 N ± 2.7 N.

During upper cervical left lateral flexion, the end ROM without C0–C1 stabilization was 5.6° ± 3.2° with a maximum force of 7.9 N ± 2.6 N. Following C0–C1 stabilization all specimens demonstrated a reduction in ROM (2.3° ± 1.2°) at all standardized forces with an average maximum force of 7.5 N ± 2.6 N.

Upper cervical transverse plane mobility. During upper cervical right axial rotation, the end ROM without C0–C1 stabilization was 33.9° ± 6.7°, with an average maximum force of 3.4 N ± 0.9 N. Following C0–C1 stabilization, all specimens demonstrated a reduction in ROM (28.5° ± 7.0°) at all standardized forces with an average maximum force of 3.9 N ± 0.7 N.

During upper cervical left axial rotation, the average end ROM without C0–C1 stabilization was 28.0° ± 6.9°, with an average maximum force of 3.8 N ± 1.4 N. All specimens demonstrated a reduction in left rotation ROM following the stabilization of C0–C1 (23.7° ± 8.5° with an average maximum force of 3.0 N ± 1.8 N) during all standardized forces except specimen 1, which had a maximum force 0.8 N lower with C0–C1 stabilization versus non-stabilization.

Table 5 shows the statistical significance of the maximal force applied and ROM at different standardized forces and end-range for non-stabilized and C0–C1 stabilization configurations in the three cardinal planes. At

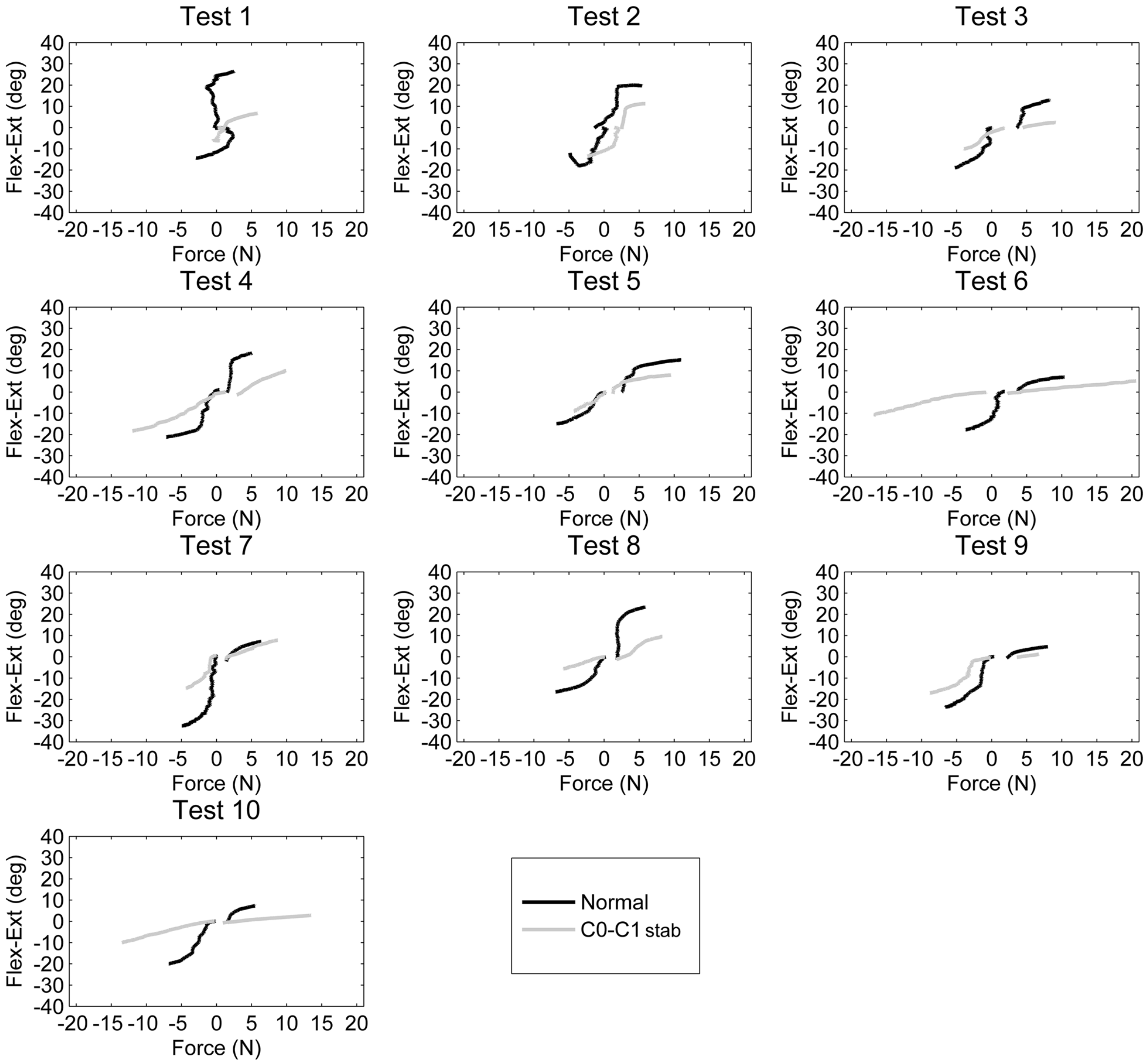

Figure 3. Forces required for flexion (negative values) and extension (positive values) during the full range of motion in the 10 specimens: normal and with C0–C1 stabilization.

the end ROM, all directions of movements showed a statistically significant reduction of movement with C0–C1 stabilization. There were no statistical differences in the maximal forces applied without and with stabilization of C0–C1 in all directions except for extension in which more force was applied with stabilization of C0–C1 ($p = 0.03$).

Discussion

To our knowledge, this is the first biomechanical study that analyzes the role of C0–C1 restriction of movement on UCS kinematics (C0–C1 and C1–C2). Screw stabilization of C0 achieved a consistent reduction of mobility in C0–C1, especially in the transverse plane.

The results of this study show that C0–C1 stabilization results in a statistically significant reduction of the ROM in the three cardinal planes. Stabilization of C0–C1 resulted in a reduction of 46.9%, 55.3%, and 15.6% in the upper cervical motion in the sagittal, frontal, and transverse plane, respectively. Also, when considering ROM with standardized forces, the stabilization of C0–C1 produced lower ROM than the non-stabilized configuration.

Sample size, age-related degenerative changes, and frequent upper cervical anatomy variations should also be considered when analyzing our results[28]. For example, anatomical variations for alar ligaments, including ligament orientation from dens to the occiput (craniocaudal, horizontal, or caudocranial)[29], variability in the origin of the ligaments on the odontoid process, and an inconsistent atlantal portion of the alar ligament[30] have been described in the literature. This inter-individual variability is likely to lead to differences in our results in the

Test		Flexion (degrees)						Extension (degrees)					
		Force						Force					
		1 N	2 N	3 N	4 N	F. Max	ROM Max	1 N	2 N	3 N	4 N	F. Max	ROM Max
1	Normal	12.7	13.8			2.9	14.5	25.1	26.0			2.5	26.6
	C0C1 stab					0.5	6.2		3.2	4.5	5.4	5.8	6.8
	Difference					−2.4	−8.3		−22.8			3.3	−19.8
2	Normal	6.5	15.2	17.6	16.6	4.9	17.9	5.8	18.7	19.7	19.9	5.4	20.0
	C0C1 stab	11.9	13.2			2.4	13.4			8.5	10.4	5.9	11.3
	Difference	5.4	−2.0			−2.5	−4.5			−11.2	−9.5	0.5	−8.7
3	Normal	9.0	13.4	15.3	16.9	5.3	18.9				1.9	8.2	12.9
	C0C1 stab	3.9	7.4	9.2		4.0	10.1					9.1	2.5
	Difference	−5.1	−6.0	−6.1		−1.3	−8.8					0.9	−10.4
4	Normal	3.2	11.7	18.1	18.9	7.1	21.2		8.9	16.1	17.4	5.0	18.5
	C0C1 stab	2.7	4.4	7.0	8.3	12.0	18.4				0.9	9.9	10.1
	Difference	−0.5	−7.3	−11.1	−10.6	4.9	−2.8				−16.5	4.9	−8.4
5	Normal	2.5	7.6	10.2	12.0	6.8	14.9			5.4	8.1	11.0	15.2
	C0C1 stab	2.9	5.8	6.7	8.6	4.3	9.0		3.0	4.9	5.6	9.5	8.0
	Difference	0.4	−1.8	−3.5	−3.4	−2.5	−5.9			−0.5	−2.5	−1.5	−7.2
6	Normal	14.4	16.0	17.2		3.7	17.7				1.8	10.4	7.0
	C0C1 stab	0.1	0.3	0.5	1.0	16.7	10.8					20.6	5.3
	Difference	−14.3	−15.7	−16.7		13.0	−6.9					10.2	−1.7
7	Normal	21.0	27.4	29.4	31.8	4.9	32.4		1.0	3.7	5.2	6.4	7.3
	C0C1 stab	6.6	10.1	12.2	14.2	4.3	14.8		0.5	1.5	2.7	8.7	7.9
	Difference	−14.4	−17.3	−17.2	−17.6	−0.6	−17.6		−0.5	−2.2	−2.5	2.3	0.6
8	Normal	4.6	10.6	12.9	14.3	6.9	16.5		15.9	20.1	21.7	5.8	23.7
	C0C1 stab	0.6	1.8	2.9	3.9	5.8	5.7			0.3	2.2	8.3	9.5
	Difference	−4.0	−8.8	−10.0	−10.4	−1.1	−10.8			−19.8	−19.5	2.5	−14.2
9	Normal	2.1	14.3	16.3	19.7	6.6	23.7			1.6	2.7	8.0	4.8
	C0C1 stab	0.8	1.5	4.4	10.2	8.8	17.0					6.7	1.2
	Difference	−1.3	−12.8	−11.9	−9.5	2.2	−6.7					−1.3	−3.6
10	Normal	0.4	5.6	11.2	16.0	6.8	20.0		3.0	5.1	6.2	5.5	7.4
	C0C1 stab	0.1	0.6	1.2	1.9	13.5	10.0			0.0	0.4	13.5	2.9
	Difference	−0.3	−5.0	−10.0	−14.1	6.7	−10.0			−5.1	−5.8	8.0	−4.5
Normal	Mean	7.6	**13.6***	**16.5***	**18.3***	5.6	**19.8***	15.4	12.2	**10.2***	**9.4***	**6.8***	**14.3***
	SD	6.6	5.9	5.6	6.0	1.5	5.2	13.6	9.7	8.0	8.0	2.6	7.7
C01 Stab	Mean	3.3	**5.0***	**5.5***	**6.9***	7.2	**11.5***		2.2	**3.3***	**3.9***	**9.8***	**6.6***
	SD	3.9	4.5	4.0	4.8	5.3	4.3		1.5	3.3	3.5	4.4	3.5
Diff	Mean	−3.8	−8.5	−10.8	−10.9	1.6	−8.2		−11.7	−7.8	−9.4	3.0	−7.7
	SD	6.7	5.6	4.7	4.8	5.1	4.1		15.8	7.9	7.2	3.8	6.1

Table 2. Flexion–extension in degrees for the force values of 1, 2, 3, and 4 N during the motion, and the maximum force (F. Max) with its range of motion (ROM Max). The table shows the values for all the specimens before (normal) and after (C0C1 stab) the stabilization of C0–C1. The means and standard deviations for each analyzed force and ROM Max are presented at the last rows of the table. N, Newtons; F max, applied force at end range of motion; ROM max, end range of motion; SD, standard deviation. *Statistical significance $p < 0.05$ values indicated in bold.

sagittal plane (Table 2), frontal plane (Table 3), and transverse plane (Table 4). For example, some specimens did not show any change during axial rotation following C0–C1 stabilization. In contrast, others showed a reduction of up to 74% during axial rotation, demonstrating a very relevant role of C0–C1 in the upper cervical rotation. Inter-individual variations are also likely to lead to differences in results.

In the sagittal plane, upper cervical movement without stabilization was 34.1° in our specimens (19.8° in flexion and 14.3° in extension). These results are similar to previously reported values of 15–25° in flexion[2] and reduced compared to the 46° reported by Ernst et al. (2015) in a sample of patients with non-specific cervicalgia. The results of Ernst et al. (2015) are not directly comparable to this study since they used an in vivo design and active motion without stabilization of C2[31].

With C0–C1 stabilization, there was a reduction of 16.0° in the sagittal plane movement. This value is similar to the average C0–C1 flexion–extension of 14–15° in most of the studies[2]. The remaining 18.1° measured

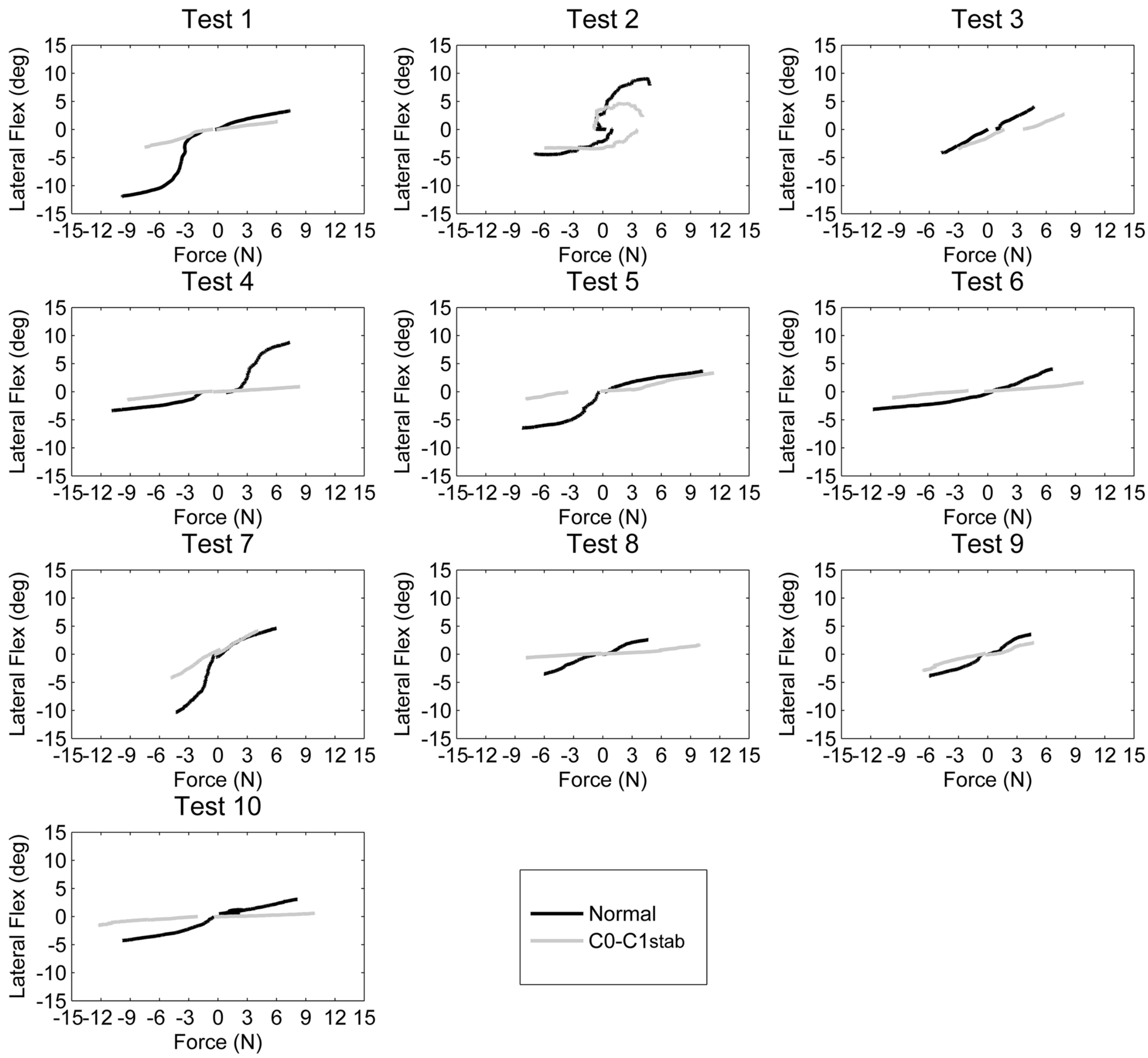

Figure 4. Forces required for left (negative values) and right lateral flexion (positive values) during the full range of motion in the 10 specimens: normal and with C0–C1 stabilization.

during C0–C1 stabilization (C1–C2 and remaining C0–C1 contribution after stabilization) are within the 10–21° reported in the literature[2].

Using the Frankfort plane as the zero position, C0–C1 accounted for 41.9% of the upper cervical flexion (or in other words: after C0–C1 stabilization, the 58.1% of the normal C0-C2 ROM was obtained). Similarly, Chancey et al. (2007) reported that C0–C1 produced 41–45% of UCS flexion. In our sample, C0–C1 produced at least 53.8% of the upper cervical extension (after C0–C1 stabilization, 46.2% of the normal C0-C2 ROM was obtained). This value differs from the reported 69–71% of the upper cervical extension occurring in C0–C1[4]. However, the C0–C1 ROM in our sample should be larger since the screw stabilization did not abolish C0–C1 ROM totally. The remaining UCS ROM after C0–C1 stabilization found within this study supports the importance of the C1–C2 segment during upper cervical flexion and extension.

In the frontal plane, upper cervical movement without stabilization was 4.7° in right lateral flexion and 5.6° in left lateral flexion. Frontal plane movements are rarely reported in the literature and are considered by some to be non-physiological movements of the atlanto-occipital joints[2]. However, motion in the frontal plane could vary between individuals. Some of the specimens in this study (1 and 7) moved approximately 15° in the frontal plane. Frequently observed anatomical variations in the upper cervical spine could explain this specimen specific movement [32].

As an average, it seems that both C0–C1 and C1–C2 participate similarly in the lateral flexion movement. During this study, C0–C1 stabilization produced 51.3% reduction in right lateral flexion and 58.0% in left lateral flexion.

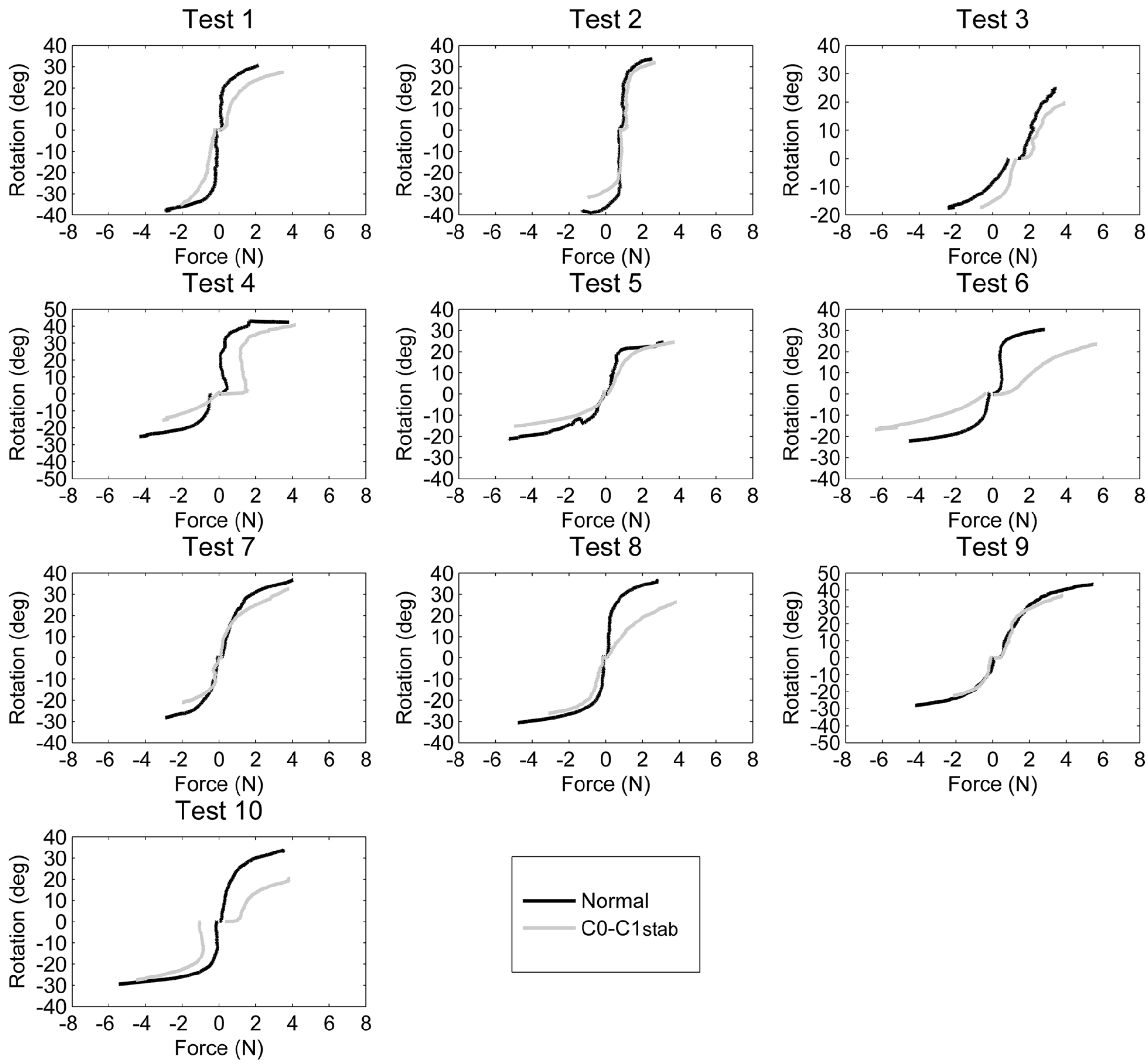

Figure 5. Forces required for left (negative values) and right axial rotation (positive values) during the full range of motion in the 10 specimens: normal and with C0–C1 stabilization.

In the transverse plane, upper cervical movement without stabilization was 61.9° (33.9° and 28.0° for right and left axial rotation, respectively). The results for upper cervical rotation are lower than the in vitro studies with reported reference values from 66.6°[33] to 92.4°[34]. This is likely due to methodological differences between this study and those previously published.

C0–C1 stabilization reduced transverse plane movement 9.8° (15.7% of the upper cervical ROM in this plane). The limitation of upper cervical axial rotation of C0–C1 in our study is similar to the in vitro studies of Panjabi et al. (1988)[34] (14.8%) and Panjabi et al. (2001)[35] (14.86%) but higher than other in vivo studies with active movements (2.4–8.9%)[9,10,36–39]. In general, it seems to be a lack of data regarding the contribution of C0–C1 to upper cervical axial rotation in the literature, and in fact, some authors even disregard it [7,9,11–13,40]. Boszczyk et al. (2012) concluded that only considering C1–C2 arthrokinematics do not explain the tolerance of the alar ligaments at the maximum of 40° of UCS rotation[12]. The findings of Boszczyk et al. suggest that C0–C1 could play a more relevant function during passive UCS rotation. In our study, C0–C1 stabilization reduced upper cervical rotatory ROM more than the reported C0–C1 range in the literature in the same direction as UCS rotation (2.5° ± 1°)[10] or even in the opposite direction as UCS rotation (– 1°)[41,42] at the end of the upper cervical rotation. Also, at 1 N, 2 N and 3 N mobilization load, the UCS rotation with C0–C1 stabilization was significantly lower than in the non-stabilized condition. The C0–C1 restriction of movement may have had an influence on alar ligament tightening. Further support for the contribution of C0–C1 during UCS axial rotation is reported clinically showing an increase of C1–C2 ROM following C0–C1 mobilization[15–19], although scientific evidence about the specific segmental effect in C0–C1 and not in adjacent segments of C0–C1 translatoric mobilization is needed.

		Right lateral flexion (degrees)						Left lateral flexion (degrees)					
		Force					ROM Max	Force					ROM Max
Test		1 N	2 N	3 N	4 N	F. Max		1 N	2 N	3 N	4 N	F. Max	
1	Normal	0.9	1.5	1.9	2.3	7.4	3.4		0.7	1.7	7.2	9.9	12.1
	C0C1 stab	0.2	0.5	0.8	0.9	6.1	1.4	0.1	0.3	1.2	1.7	7.5	3.2
	Difference	−0.7	−1.0	−1.1	−1.4	−1.3	−2.0		−0.4	−0.5	−5.5	−2.4	−8.9
2	Normal	6.0	7.7	8.5	8.9	4.8	9.0	2.7	3.2	3.9	4.2	7.0	4.5
	C0C1 stab	3.8	4.5	4.1	2.4	4.1	4.7	3.4	3.4	3.3	3.3	6.0	3.4
	Difference	−2.2	−3.2	−4.4	−6.5	−0.7	−4.3	0.7	0.2	−0.6	−0.9	−1.0	−1.1
3	Normal	0.2	1.5	2.4	3.2	4.7	4.1	0.8	1.9	2.9	3.7	4.8	4.1
	C0C1 stab				0.2	7.8	2.7	2.1	2.7	3.4		3.0	3.4
	Difference				−3.0	3.1	−1.4	1.3	0.8	0.5		−1.8	−0.7
4	Normal	0.0	0.3	2.6	5.4	7.4	8.8		0.5	1.4	1.8	10.9	3.4
	C0C1 stab	0.1	0.2	0.3	0.4	8.4	0.9		0.1	0.2	0.3	9.3	1.4
	Difference	0.1	−0.1	−2.3	−5.0	1.0	−7.9		−0.4	−1.2	−1.5	−1.6	−2.0
5	Normal	0.7	1.3	1.7	2.1	10.2	3.7	1.7	3.3	4.8	5.4	8.3	6.5
	C0C1 stab	0.2	0.3	0.4	0.6	11.4	3.4				0.2	7.9	1.3
	Difference	−0.5	−1.0	−1.3	−1.5	1.2	−0.3				−5.2	−0.4	−5.2
6	Normal	0.5	0.9	1.4	2.3	6.7	4.1	0.8	1.1	1.5	1.8	11.8	3.1
	C0C1 stab	0.2	0.3	0.5	0.6	9.8	1.7				0.1	9.8	1.1
	Difference	−0.3	−0.6	−0.9	−1.7	3.1	−2.4				−1.7	−2.0	−2.0
7	Normal	1.0	2.2	3.0	3.6	6.0	4.6	3.3	7.0	8.7	10.0	4.3	10.4
	C0C1 stab	1.2	2.3	3.2	4.1	4.1	4.2	0.2	1.3	2.5	3.6	4.8	4.3
	Difference	0.2	0.1	0.2	0.5	−1.9	−0.4	−3.1	−5.7	−6.2	−6.4	0.5	−6.1
8	Normal	0.4	1.5	2.1	2.4	4.7	2.6	0.2	0.7	1.5	2.3	6.0	3.5
	C0C1 stab	0.1	0.2	0.3	0.4	9.9	1.7			0.1	0.2	7.9	0.7
	Difference	−0.3	−1.3	−1.8	−2.0	5.2	−0.9			−1.4	−2.1	1.9	−2.8
9	Normal	0.4	1.9	2.9	3.4	4.5	3.5	0.9	1.9	2.5	3.0	6.0	3.9
	C0C1 stab	0.1	0.6	1.4	1.8	4.7	2.1	0.1	0.5	0.8	1.3	6.6	2.9
	Difference	−0.3	−1.3	−1.5	−1.6	0.2	−1.4	−0.8	−1.4	−1.7	−1.7	0.6	−1.0
10	Normal	0.7	1.2	1.3	1.6	8.1	3.1	0.8	1.6	2.2	2.7	9.8	4.3
	C0C1 stab	0.0	0.1	0.1	0.1	9.9	0.6			0.1	0.2	12.2	1.5
	Difference	−0.7	−1.1	−1.2	−1.5	1.8	−2.5			−2.1	−2.5	2.4	−2.8
Normal	Mean	**1.1***	**2.0***	**2.8***	**3.5***	6.5	**4.7***	1.4	2.2	**3.1***	**4.2***	7.9	**5.6***
	SD	1.8	2.1	2.1	2.2	1.9	2.3	1.1	2.0	2.3	2.6	2.6	3.2
C0C1 Stab	Mean	**0.7***	**1.0***	**1.2***	**1.1***	7.6	**2.3***	1.2	1.4	**1.5***	**1.2***	7.5	**2.3***
	SD	1.2	1.5	1.4	1.3	2.7	1.4	1.5	1.4	1.4	1.4	2.6	1.2
Diff	Mean	−0.5	−1.1	−1.6	−2.4	1.2	−2.4	−0.5	−1.2	−1.7	−3.1	−0.4	−3.3
	SD	0.7	0.9	1.3	2.0	2.2	2.3	2.0	2.3	2.0	2.1	1.7	2.7

Table 3. Lateral flexion in degrees for the force values of 1, 2, 3, and 4 N during the motion, and the maximum force (F. Max) with its range of motion (ROM Max). The table shows the values for all the specimens before (normal) and after (C0C1 stab) the stabilization of C0–C1. The means and standard deviations for each analyzed force and ROM Max are presented at the last rows of the table. N, Newtons; F max, applied force at end range of motion; ROM max, end range of motion; SD, standard deviation. *Statistical significance $p < 0.05$ values indicated in bold.

The alar ligaments are considered a primary restraint to axial rotation[22,43,44]. Findings from this study indicate there is a reduction of the upper cervical axial rotation ROM and increased forces when C0–C1 is stabilized compared to non-stabilization. This increase of resistance in upper cervical axial rotation with C0–C1 stabilization could mean that C0–C1 kinematics are related to the tightening of the alar ligaments and indirectly, to the upper cervical and C1–C2 ROM in the transverse plane. In fact, research investigating the impact of the alar ligament on upper cervical axial rotation indicate that alar ligament transection increases C0–C1 axial rotation by 30%[37].

The data from our study provides insight into the effect of surgical applications of treating C0–C1 dislocation via C0–C1 transcondylar screw techniques[45–47]. We observed a ROM reduction after C0–C1 stabilization in each plane as happens with the surgical insertion of transarticular screws. It is known that adding a structural graft may further improve the amount of stability in the C0–C1 segment[46]. However, the results of this study are not directly comparable to the typical surgical procedure because of the different fixating method and the different entry point and screw's trajectory from the in vivo techniques. Even with these differences in stabilization

Test		Right rotation (degrees)						Left rotation (degrees)					
		Force						Force					
		1 N	2 N	3 N	4 N	F. Max	ROM Max	1 N	2 N	3 N	4 N	F. Max	ROM Max
1	Normal	26.1	30.0			2.2	30.8	33.6	36.0			2.9	38.0
	C0C1 stab	16.6	23.5	26.3		3.5	27.3	26.1	34.9			2.1	41.9
	Difference	−9.5	−6.5			1.3	−3.5	−7.5	−1.1			−0.8	3.9
2	Normal	5.7	32.6			2.5	33.5	38.5				1.3	39.0
	C0C1 stab	0.4	30.0			2.7	31.9					1.0	31.9
	Difference	−5.3	−2.6			0.2	−1.6					−0.3	−7.1
3	Normal	0.0	7.5	21.8		3.5	24.8	13.6	16.4			2.5	17.6
	C0C1 stab		1.0	15.9		3.9	19.8					0.7	17.3
	Difference		−6.5	−5.9		0.4	−5.0					−1.8	−0.3
4	Normal	38.0	42.7	42.5		3.8	43.9	15.6	20.7	22.7	24.4	4.3	25.4
	C0C1 stab	0.4	34.9	38.3	40.3	4.2	40.9	7.1	11.4	15.2		3.1	15.7
	Difference	−37.6	−7.8	−4.2		0.4	−3.0	−8.5	−9.3	−7.5		−1.2	−9.7
5	Normal	21.3	22.1	24.2		3.1	24.6	12.2	14.5	17.8	19.4	5.3	21.1
	C0C1 stab	15.0	21.3	23.3		3.7	24.5	7.9	11.0	12.6	14.0	5.0	15.2
	Difference	−6.3	−0.8	−0.9		0.6	−0.1	−4.3	−3.5	−5.2	−5.4	−0.3	−5.9
6	Normal	26.7	29.2			2.8	30.5	15.1	18.8	20.4	21.6	4.6	22.1
	C0C1 stab	1.3	8.9	15.3	19.5	5.7	23.6	3.5	7.5	10.4	12.7	6.4	17.0
	Difference	−25.4	−20.3			2.9	−6.9	−11.6	−11.3	−10.0	−8.9	1.8	−5.2
7	Normal	22.7	31.0	34.1	36.7	4.0	36.9	21.5	26.3			2.9	28.4
	C0C1 stab	19.6	24.9	29.4		3.8	32.8	18.0				2.0	21.1
	Difference	−3.1	−6.1	−4.7		−0.2	−4.1	−3.5				−0.9	−7.3
8	Normal	30.0	34.3			2.9	36.5	23.2	26.5	28.4	29.6	4.8	30.8
	C0C1 Stab	11.4	19.2	23.4		3.9	26.5	19.8	24.3	26.0		3.1	26.3
	Difference	−18.6	−15.1			1.0	−10.0	−3.4	−2.2	−2.4		−1.7	−4.5
9	Normal	16.1	31.1	37.6	40.4	5.5	43.5	18.9	23.7	26.0	27.7	4.2	28.0
	C0C1 Stab	18.7	28.9	33.7		3.8	36.8	18.3	22.0			2.2	22.5
	Difference	2.6	−2.2	−3.9		−1.7	−6.7	−0.6	−1.7			−2.0	−5.5
10	Normal	24.3	30.1	32.5		3.6	33.7	23.4	26.0	27.3	28.2	5.5	29.5
	C0C1 stab	0.8	13.6	17.5		3.8	20.5	16.9	22.5	24.9	26.7	4.5	27.6
	Difference	−23.5	−16.5	−15.0		0.2	−13.2	−6.5	−3.5	−2.4	−1.5	−1.0	−1.9
Normal	Mean	**21.1**	**29.1***	**32.1***	38.6	3.4	**33.9***	**21.6***	**23.2***	**23.8***	25.2	3.8	**28.0***
	SD	11.3	9.1	7.9	2.6	0.9	6.7	8.6	6.5	4.2	4.0	1.4	6.9
C0C1 stab	Mean	**9.4***	**20.6***	**24.8***	29.9	3.9	**28.5***	**14.7***	**19.1***	**17.8***	17.8	3.0	**23.7***
	SD	8.5	10.3	8.0	14.7	0.7	7.0	7.7	9.6	7.2	7.7	1.8	8.5
Diff	Mean	−14.1	−8.4	−5.8		0.5	−5.4	−5.7	−4.7	−5.5	−5.3	−0.8	−4.4
	SD	13.0	6.6	4.8		1.2	3.9	3.5	4.0	3.3	3.7	1.1	3.9

Table 4. Rotation in degrees for the force values of 1, 2, 3, and 4 N during the motion, and the maximum force (F. Max) with its range of motion (ROM Max). The table shows the values for all the specimens before (normal) and after (C0C1 stab) the stabilization of C0–C1. The means and standard deviations for each analyzed force and ROM Max are presented at the last rows of the table. N: Newtons; F max: applied force at end range of motion; ROM max: end range of motion; SD: standard deviation. *Statistical significance $p < 0.05$ values indicated in bold.

methods, this study provides valuable 3D motion and load information during a simulated manual clinical procedure used to examine upper cervical kinematics.

Other limitations of the present study relate to the mobilization procedure. The methodology used was original and specific to the objectives but challenging to compare with prior studies. The in vitro design allowed the stabilization of C2 as a fixed point for movement reference. The mobilization force was manually applied to simulate a clinical and physiological procedure in comparison to loading devices. Inducing the mobilization manually challenges the repeatability in terms of direction and magnitude of the loads. However, after the experimental testing, the study compared certain force values (1 N, 2 N, 3 N, and 4 N) in each of the planes in both conditions. Physiological motion can also be produced by machines[34]. However, intersegmental movement outside the primary plane of motion (coupled motions) has also been reported in experimental testing using machines loading in one anatomical plane[48]. Also, the structures dissected before the applied movements[49] may also influence the results.

	Flexion		Extension		Right lateral flexion		Left lateral flexion		Right rotation		Left rotation	
	Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value
1N_Normal	7.6 ± 6.6	0.110	15.4 ± 13.6	–	1.1 ± 1.8	**0.021**	1.4 ± 1.1	0.715	21.1 ± 11.3	**0.011**	21.6 ± 8.6	**0.012**
1N_Stabilized	3.3 ± 3.9		-		0.7 ± 1.2		1.2 ± 1.5		9.4 ± 8.5		14.7 ± 7.7	
2N_Normal	13.6 ± 5.9	**0.008**	12.2 ± 9.7	0.180	2.0 ± 2.1	**0.011**	2.2 ± 2.0	0.249	29.1 ± 9.1	**0.005**	23.2 ± 6.5	**0.018**
2N_Stabilized	5.0 ± 4.5		2.2 ± 1.5		1.0 ± 1.5		1.4 ± 1.4		20.6 ± 10.3		19.1 ± 9.6	
3N_Normal	16.5 ± 5.6	**0.012**	10.2 ± 8.0	**0.043**	2.8 ± 2.1	**0.011**	3.1 ± 2.3	**0.021**	32.1 ± 7.9	**0.028**	23.8 ± 4.2	**0.043**
3N_Stabilized	5.5 ± 4.0		3.3 ± 3.3		1.2 ± 1.4		1.5 ± 1.4		24.8 ± 8.0		17.8 ± 7.2	
4N_Normal	18.3 ± 6.0	**0.028**	9.4 ± 8.0	**0.028**	3.5 ± 2.2	**0.007**	4.2 ± 2.6	**0.008**	38.6 ± 2.6	–	25.2 ± 4.0	0.109
4N_Stabilized	6.9 ± 4.8		3.9 ± 3.5		1.1 ± 1.3		1.2 ± 1.4		29.9 ± 14.7		17.8 ± 7.7	
NMax_Normal	5.6 ± 1.5	0.721	6.8 ± 2.6	**0.037**	6.5 ± 1.9	0.169	7.9 ± 2.6	0.575	3.4 ± 0.9	0.093	3.8 ± 1.4	0.059
NMax_Stabilized	7.2 ± 5.3		9.8 ± 4.4		7.6 ± 2.7		7.5 ± 2.6		3.9 ± 0.7		3.0 ± 1.8	
ROMMax_Normal	19.8 ± 5.2	**0.005**	14.3 ± 7.7	**0.007**	4.7 ± 2.3	**0.005**	5.6 ± 3.2	**0.005**	33.9 ± 6.7	**0.005**	28.0 ± 6.9	**0.013**
ROMMax_Stabilizd	11.5 ± 4.3		6.6 ± 3.5		2.3 ± 1.4		2.3 ± 1.2		28.5 ± 7.0		23.7 ± 8.5	

Table 5. Statistical significance of the maximal force applied and range of motion (degrees) at different standardized forces and end-range for normal and stabilized C0–C1 configurations in flexion, extension, right lateral flexion, left lateral flexion, right axial rotation, and left axial rotation. N: Newtons; F max: applied force at end range of motion; ROM max: end range of motion; SD: standard deviation. *p* values in bold showed statistical significance ($p < 0.05$).

This in vitro study, showed a reduction in all cardinal plane motions following stabilization of C0–C1. During transverse plane motion, C0–C1 stabilization reduced upper cervical rotation by 15%, a higher rate than expected, considering the reported C0–C1 rotational range of movement in the literature. In addition, the increase of resistance in upper cervical axial rotation with C0–C1 stabilization could mean that C0–C1 kinematics could be related to the tightening of the alar ligaments.

Received: 29 October 2020; Accepted: 5 May 2021
Published online: 25 May 2021

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Acknowledgements
The authors are thankful to body donors that enable the study of anatomy. Thanks to the Instituto Aragonés de Fomento (IAF), Unidad de Investigación en Fisioterapia at University of Zaragoza, Anatomy Laboratories from Universitat Internacional de Catalunya and the Ethical Committee from UIC-Barcelona for its support. And we also thank to Judith Pascual and Dr. Pedro Alvarez for their help in the anatomy preparation.

Author contributions
Conceptualization: C.H.G., A.I.L., O.L.L., J.M.T.; Methodology: C.H.G., A.I.L., M.M.F., J.K., A.P.B.; Formal analysis and investigation: C.H.G., A.I.L., M.M.U., J.M.T., M.M.F., J.K.; Writing—original draft preparation: C.H.G., A.I.L., C.L.C., M.M.F.; Writing—review and editing: C.H.G., A.I.L., C.L.C., O.L.L., M.M.U., J.R.S., M.M.F., J.M.T., J.K., A.P.B.; Funding acquisition: C.H.G. M.M.F., J.M.T., A.P.B., O.L.L.; Resources: M.M.F., J.M.T., A.P.B.; Supervision: C.H.G., A.I.L., O.L.L., M.M.F., J.K., A.P.B., J.R.S., M.M.F., J.M.T., M.M.U.

Funding
This study was supported by IAF (Instituto Aragonés de Fomento).

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to C.H.-G.

Chapter 7

Discussion

This thesis is based on four research articles already published in different journals. These four articles address the upper cervical spine kinematics in the three anatomical planes (sagittal, frontal, and transverse) considering the skull (C0) and the first two uppermost cervical vertebrae: atlas (C1) and axis (C2). A total of ten C0-C2 specimens were manually mobilized, measuring the intersegmental range of motion (ROM) as well as the load applied by the operator, a manual physiotherapist.

Although the kinematics of the upper cervical spine have been studied over the last decades, several unknowns are still present nowadays in biomechanical studies as well as in regular clinical practices. Numerous basic science investigations have been focused only on cervical spine kinematics with a vast number of different methodologies: in vivo with healthy asymptomatic populations (Zhao et al., 2013; Anderst, 2015) or patients (Takeshima et al., 2002; Patijn et al., 2001), with mathematical models (Crisco et al., 1991b; Boszczyk et al., 2012) or models for computational simulations (Fice and Cronin, 2012; Gupta et al., 2020), and with human specimens in cadaveric studies (Panjabi et al., 2001a; Cattrysse et al., 2007a). It is also possible to find studies about the upper cervical spine kinematics oriented toward manual therapy techniques, both in vivo (Osmotherly et al., 2013a) and with human specimens (Cattrysse et al., 2008), as the experimental approach of the present thesis. Lastly, always when a clinical concern is addressed, its clinical conditions are reproduced as similarly as possible. This goal of reaching the closest conditions to the clinics explains the setup of the experimental tests herein presented: manual mobilization and continuous loading (Chapter 2, *Material and Methods*).

In this chapter, the main findings of the four articles of this thesis are described (Section 7.1). Afterwards, the experimental test protocol of this thesis is explained, comparing it with other experimental setups founds in the literature (Section 7.2).

Once the information about the different methodologies has been provided, the cervical spine kinematics and the variety of results found in the literature are discussed (Section 7.3). The clinical implications of the cadaveric tests conducted in this thesis are reported (Section 7.4).

After the discussion of the previously mentioned topics, it is possible to read about the strengths and the limitations of the in vitro work of this thesis (Section 7.5) and the scientific dissemination conducted during its years (Section 7.6). In the same research line of these studies, future work with new in vitro tests has been already planned and scheduled (Section 7.7). Lastly, the conclusions of this thesis are presented (Subsection 7.8).

7.1 Main Findings

This section summarizes the findings of each of the four articles which form this thesis, being divided in one subsection per article.

7.1.1 The alar ligament and the side-bending stress test

The article *Effect of Alar Ligament Transection in Side-bending Stress Test: a Cadaveric Study* (Chapter 3) presents the increase in lateral bending ROM after unilateral transection of the alar ligament. Although only the right side of the alar ligament was cut, the motion increased in both directions, right (33.5% or $1.30 \pm 1.54°$) and left (27.5% or $1.88 \pm 1.51°$). Nevertheless, this increase in the lateral bending motion was not observed to the right in one specimen and to the left in another, which means that the side-bending stress test would not have been sensitive to the alar ligament transection in all the specimens by only assessing the lateral bending ROM. From a clinical point of view, another remark is the value of the ROM increase after the ligament transection, which is in most of the cases below 2°; this increase may not be easily detected by practitioners in the side-bending stress test.

This low increase in the ROM after alar ligament transection supports the consideration of the resistance perceived in side-bending stress test and the importance that the resistance might have over the ROM when diagnosing instability. Nevertheless, the ROM quantified before the alar ligament transection is of high value from a clinical perspective, as C0-C2 lateral bending has been related to ligaments damage when it occurs in patients. Lastly, the lateral bending observed prior to ligament transection suggests that the alar ligaments are not fully tight when the craniovertebral junction is in neutral position.

7.1.2 The alar ligament and the rotation stress test

The article *The Effect of Alar Ligament Transection on the Rotation Stress Test: a Cadaveric Study* (Chapter 4) quantified the axial rotation before and after unilateral alar ligament transection. After cutting the right side of the alar ligament, the axial rotation ROM increased in both sides, right (13.7% or 4.6 $\pm$ 4.9°) and left (12.9% or 3.6 $\pm$ 3.9°). Only one specimen reduced the ROM to the right side after the transection, and a different specimen reduced the ROM to the left side. The nine remaining specimens showed an increase in the axial rotation ROM, but the values of the increase were different among the specimens. This inter-subject variability pointed out the importance that anatomical variations can have among patients.

As it happened in the previous section (Section 7.1.1), the reduced resistance in axial rotation after alar ligament transection seems to be a parameter to be considered in instability clinical assessments. This concept has been previously introduced in the literature (Dvorak and Panjabi, 1987; Kaale et al., 2008).

7.1.3 Intersegmental kinematics: before and after alar ligament transection

The article *Intersegmental Kinematics of the Upper Cervical Spine: Normal Range of Motion and its Alteration After Alar Ligament Transection* (Chapter 5) addresses the change in the intersegmental ROM (C0-C1 and C1-C2), in the three anatomical planes (sagittal, frontal, and transverse), after unilateral alar ligament transection. The study of the kinematics at this intersegmental level allows a better understanding of the kinematics at the occipito-atlanto-axial joint complex.

In the sagittal plane (flexion-extension motion), the motion increased after alar ligament transection. The motion increased in both directions, flexion (15.5% or 2.9 $\pm$ 4.9°) and extension (15.6% or 2.2 $\pm$ 6.4°). This increase was observed in all but two specimens for the extension motion and in one specimen for the flexion motion. Considering all the specimens, the mean value of the C0-C2 ROM increased, although the C0-C1 flexion ROM and the C1-C2 extension ROM decreased.

In the frontal plane (lateral bending motion), the C0-C2 ROM increased after alar ligament transection, as described in Section 7.1.1. However, the C1-C2 right lateral bending only showed a slightly increase after the transection (0.2°; from 1.6 $\pm$ 1.2° to 1.8 $\pm$ 1.1°). In the rest of the cases (C0-C1 and C1-C2, right and left) the increase averages were between 0.8° and 1.2°.

In the transverse plane (axial rotation motion), the C0-C2 ROM increased after alar ligament transection, as described in Section 7.1.2. In this plane, C1-C2 maintained its ROM to the right side after the ligament transection, showing an increase of only 0.1° (from 32.3 $\pm$ 9.3° to 32.4 $\pm$ 9.4°). However, a slightly higher increase was observed in C0-C1 for the right side (2.0° for the right side and 1.1° for the left side). Related to the axial rotation, the C0-C1 rotation occurred in the same direction as the head in most of the specimens, and none of them showed a C0-C1 rotation to the opposite side of the head's rotation for right and left sides, as previous studies have observed (Penning and Wilmink, 1987; Iai et al., 1993).

As it was mentioned in the two previous sections (Sections 7.1.1 and 7.1.2), and as the standard deviations presented in this section show: inter-subject variability was observed in the three analyzed planes.

7.1.4 Occipital-atlas stabilization

The article *Effects of Occipital-Atlas Stabilization in the Upper Cervical Spine Kinematics: an In Vitro Study* (Chapter 6) presents how the motion of the C0-C1 segment influences the upper cervical spine kinematics (C0-C2). The motion was studied in the three anatomical planes (sagittal, frontal, and transverse), and, after C0-C1 stabilization, the C0-C2 motion was reduced in the three planes: 46.9% in the sagittal plane, 55.3% in the frontal plane, and 15.6% in the transverse plane.

In the sagittal plane (flexion-extension motion), all the specimens decreased the flexion ROM after C0-C1 stabilization. The average flexion of all the specimens went from $19.8 \pm 5.2°$ to $11.5 \pm 4.3°$. In extension, after the C0-C1 stabilization, C0-C2 motion decreased in all the specimens except in one. The average extension decreased from $14.3 \pm 7.7°$ to $6.6 \pm 3.5°$.

In the frontal plane (lateral bending motion), all the specimens reduced their range of motion to both sides after the C0-C1 stabilization: the right side went from $4.7 \pm 2.3°$ to $2.3 \pm 1.4°$, and the left side went from $5.6 \pm 3.2°$ to $2.3 \pm 1.2°$.

In the transverse plane (axial rotation motion), all the specimen reduced their axial rotation to the right side and all but one reduced it to the left side. The decrease to the right side was from $33.9 \pm 6.7\,°$ to $28.5 \pm 7.0°$, and in the left side the change was from $28.0 \pm 6.9°$ to $23.7 \pm 8.5°$.

The applied load after the C0-C1 stabilization increased in the sagittal plane (from 5.6 ± 1.5 N to 7.2 ± 5.3 N in flexion; from 6.8 ± 2.6 N to 9.8 ± 4.4 N in extension). Despite of this increase, the flexion and extension ROM decreased after the C0-C1 stabilization. In the frontal plane this increase in the applied load was not observed. For the right side, the load went up from 6.5 ± 1.9 N to 7.6 ± 2.7 N, while in the left side the average load after the stabilization slightly decreased from 7.9 ± 2.6 N to 7.5 ± 2.6 N. Lastly, a similar response was observed in the transverse plane: an increase in the right side (from 3.4 ± 0.9 N to 3.9 ± 0.7 N) but a reduction in the left side (from 3.8 ± 1.4 N to 3.0 ± 1.8 N).

When reviewing the results of this paper, it must be considered that the C0-C1 movement was not fully restricted by the screw stabilization. With the C0-C1 stabilization, the C0-C1 ROM was restricted by 74.4% in the sagittal plane, 76.9% in the frontal plane, and 90.9% in the transverse plane.

7.2 Experimental Test Protocol

Different in vitro methodologies are described in the literature. Therefore, this section aims to collect the main differences related to the specimens, the motion analysis, and the loading conditions, to better understand the in vitro protocol designed in this thesis (Chapter 2, *Material and Methods*).

7.2.1 In vitro and in vivo tests

The extrapolation of in vitro observations to in vivo situations has been commonly found in the literature for decades (Goel et al., 1988; Wilke et al., 1998a). The mechanical behavior of the specimens is preserved after their storage in frozen conditions (Panjabi et al., 1985b). However, it has been stated that the movement in cadavers is less accurate in comparison to in vivo studies (Bogduk and Mercer, 2000).

The downside of using cadavers is mainly due to their lack of musculature (Bogduk and Mercer, 2000), but other reasons have been stated:

- **Removing soft tissues** in cadavers might increase the mobility and overestimate in vivo ROM (Buzzatti et al., 2015).
- **The lack of compressive forces** in specimens decreases their biofidelity to represent the in vivo load-displacement response (Panjabi et al., 2001b).
- Manual therapy techniques applied on cadavers are **more aggressive than on patients**: cadavers receive higher preload and peak force during mobilizations, and in shorter times (Symons et al., 2012).
- During the simulation of clinical techniques on cadavers, an important factor might be the absence of **the body reactions of the patients and their verbal feedback** (Gianola et al., 2015).
- Sometimes the manual techniques simulated with cadavers require a **grip of a vertebra**, the grip in cadavers can be more direct when the soft tissues have been removed. Therefore, the grip in cadavers restricts more the mobility than the same grip in patients (Gianola et al., 2015).
- **The positioning** of the specimens can influence on the kinematics response, e.g., kinematics measurements on vertically downwards specimens, which have the skull below the vertebrae, missed the load from the skull to the spinal segments (Dugailly et al., 2009). But most of the specimens are positioned in a natural upright position (Panjabi et al., 1991b; Panjabi et al., 1988; Tisherman et al., 2020). The specimens have also been placed horizontally when clinical manual techniques are in vitro simulated (Cattrysse et al., 2007a; Cattrysse et al., 2007b).

Nevertheless, Kettler et al., 2002, compared the upper cervical spine kinematics with and without mechanically **simulated muscle forces**, and reached the conclusion that no overestimation of spinal instability appears without muscles simulations when the results are compared with the same condition without the use of artificial muscles. Furthermore, few decades ago, in vitro tests were the only methodology to accurately study 3D intersegmental kinematics in a controlled environment (Panjabi et al., 2001a). Nowadays, it is possible to study 3D intersegmental kinematics with in vivo protocols (Ishii et al., 2004; Salem et al., 2013; Anderst et al., 2017; Zhou et al., 2020) or computational models (Panzer et al., 2011).

This thesis has required the use of specimens to replicate alar ligament injuries and compare the same specimens with and without the alar ligament injury. The use of cadavers to cause injuries and compare with their intact conditions has been presented in previous investigations, and also with alar ligament transections (Dvorak et al., 1987b; Panjabi et al., 1991a; Panjabi et al., 1991b; Kettler et al., 2002; Tisherman et al., 2020).

7.2.2 Length of the specimens

Among the in vitro configurations, one of the main differences is the segments included in the specimens. Some ROM analyses are only with one cervical segment, including only two vertebrae (Nightingale et al., 2007; Barker et al., 2014; Muth-seng et al., 2019). However, most of the studies include several vertebrae, but varying the cervical levels considered:

- **C0-C2**: Nightingale et al., 2007.
- **C0-C3**: Dvorak et al., 1987a; Panjabi et al., 1991a; Crisco et al., 1991a; Nightingale et al., 2007; Dugailly et al., 2009; Chin et al., 2003; Tisherman et al., 2020.
- **C0-C5**: Goel et al., 1988; Kettler et al., 2002.
- **C0-C7**: Panjabi et al., 1988; Hartwig et al., 2004.
- **C0-T1**: Cattrysse et al., 2007a; Panjabi et al., 2001b.

Having different vertebral levels complicates the comparison of kinematics between different studies, as seen in the next section (Section 7.3, *Upper Cervical Spine Kinematics*). Moreover, the specimens with less segments violate the continuity of the full cervical spine, without representing the cervical lordosis and lacking the longitudinal ligaments (Panjabi et al., 2001a; Dickey and Kerr, 2003).

In this thesis, all the experimental tests have been conducted on C0-C2 specimens. Having only these uppermost vertebral levels facilitate the quantification of the upper cervical spine ROM, which show a wide range in the literature (Section 7.3, *Upper Cervical Spine Kinematics*). Furthermore, the goal of testing how the alar ligaments influence the kinematics of the upper cervical spine does not require the levels below C2 (Chapters 3, 4, and 5). The goal of studying the upper cervical spine with restricted C0-C1 mobility was also conducted with C0-C2 specimens (Chapter 6). Moreover, the clinical mobilizations simulated in the in vitro tests were performed using a grip on C2 to stabilize this vertebral level (Osmotherly et al., 2013a); therefore, having the C2 vertebra as the lowest level in the specimens, and rigidly fixed, helped to replicate the grip of C2.

7.2.3 Initial positioning of the specimens

To quantify the kinematics of the spine, an initial position must be defined and taken as a reference. This provide a similar starting position for all the specimens.

Commonly, a **neutral position** is applied, but sometimes the definition of this position is missing (Panjabi et al., 2001a; Buzzatti et al., 2015). With spine specimens fixed on a loading machine, this neutral position has been defined as the unloaded configuration (Caravaggi et al., 2017). A similar definition has been used during in vitro manual mobilizations, mentioning the manual feel of the operator where the least passive tension is perceived (Dugailly et al., 2009). However, this definition of neutral position considering only the applied tension might be influenced by injuries. The position without load is sensitive to spinal injuries (Oxland and Panjabi, 1992).

When the experimental tests have **in vivo** scenarios, other definitions for the initial position (or neutral position) are found. In these cases, the neutral reference is understood as a relaxed position with the head looking forward (Lind et al., 1989). But anatomical references are sometimes considered, such as the angle between the line joining the foramena lacerum (skull) and the line joining the transverse foramena of C1 (Osmotherly et al., 2013a).

Anatomical references were used in the tests of this thesis. First, considering **the Frankfurt plane**, which is based on the infraorbital foramen and the external auditory meatus (Moorrees and Kean, 1958), a line from the right infraorbital foramen to the right external auditory meatus was aligned to an horizontal reference from a laser. Second, other laser was used to control the vertical position of the skull, with a vertical line in the center of the face (Chapter *Material and Methods*, Section 2.3). This technique with **the use of two lasers**, or any other similar technique considering anatomical landmarks, has not been found in any of the other in vitro studies focused on the upper cervical spine kinematics (Table 7.1).

7.2.4 Motion quantification

To quantify vertebral kinematics, a wide variety of methodologies is described in the literature. This subsection focuses on methodologies applied to in vitro configurations, as the protocol followed in this thesis has only in vitro tests.

The following methodologies to quantify the motion of the upper cervical spine have been applied in previous in vitro test:

- **Computer tomography** (CT): Dvorak et al., 1987b.
- **Optoelectronic devices with infrared light** tracked by two cameras: Goel et al., 1988, with an accuracy of 0.6°.
- **Stereophotogrammetric system**, using X-ray images to determine the position of the markers with respect to the vertebrae: Panjabi et al., 1988, with an accuracy of less than 0.5°, Panjabi et al., 1991a, with an accuracy of 0.37°, and Panjabi et al., 2001a, with an accuracy between 0.17° and 0.60°, as it was different in each anatomical plane.
- **Optical markers** tracked by a digital camera system: Nightingale et al., 2007, with an accuracy of less than 0.1°.
- **Rotatory potentiometer**: Kettler et al., 2002, with an accuracy of less than 0.01°.
- **3D location of markers and 3D models** from CT images: Dugailly et al., 2009, with an error of 0.125–0.3 mm.
- **Reflective markers** and cameras which emit and track infrared light: Chin et al., 2003, and Yoganandan et al., 2007 (accuracy of 0.1°), both studies use a Vicon system (Vicon, Oxford, United Kingdom).

The wide variety of possible methodologies and the limited number of in vitro studies focused on the upper cervical spine make that some of the mentioned techniques have only been found in one study. However, some of these techniques are very similar and have the same physical concept behind, such as the devices with infrared light tracked by cameras and the cameras with infrared light which track passive reflective markers (Goel et al., 1988; Chin et al., 2003; Yoganandan et al., 2007).

In the experimental test of this thesis, as in the last methodology enumerated, a **Vicon system** tracked the motion. The system had four cameras and markers were fixed to the skull, C1, and the load cell, where C2 was rigidly attached (Chapter *Material and Methods*, Section 2.3).

To determine the position of the markers with respect to the bones where they are attached, Panjabi et al., 1985a, took X-ray images of the specimens with the markers, and this methodology was applied in several studies focused on the upper cervical spine kinematics (Crisco et al., 1991a; Panjabi et al., 1991b; Panjabi et al., 1991a; Panjabi et al., 1988; Panjabi et al., 2001a). A **3D measuring device** (FaroArm, FARO Technologies, Lake Mary, FL, USA) has been used for this same purpose of knowing the position of the markers with respect to the bones (Figure

7.1). The steps to know the Vicon markers with respect to the vertebrae are explained in Chapter *Material and Methods* (Section 2.3). This same device has also been used in previous studies to know the 3D coordinates of markers attached to the vertebrae of the upper cervical spine (Dugailly et al., 2009).

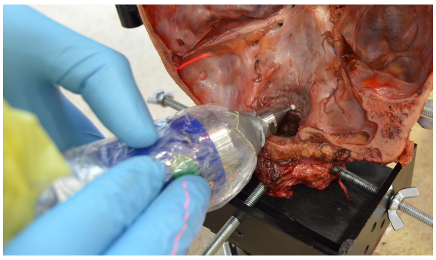

FIGURE 7.1: Measurement of the 3D coordinates of anatomical landmarks and Vicon markers to know the position of the markers with respect to the bones.

7.2.5 Loading cervical specimens

The experimental protocols to tests cervical spine specimens vary widely (Zhang et al., 2020). The specimens in previous in vitro tests have been loaded applying **a continuous load or a stepwise load**. This difference in the in vitro protocol influences the range of motion quantified in the specimens: larger motions are obtained with the stepwise loading (Goertzen et al., 2004). Furthermore, the **loading rate** also influences the behavior of the specimens: rates between 0.5°/s and 5.0°/s are recommended because slower rates produce creep effects and faster rates have consequences due to inertial effects (Wilke et al., 1998b; Wilke et al., 1998a). Loading with much faster rates (up to 2033°/s), the stiffness of the specimens is highly increased (Voo et al., 1998; Panjabi et al., 1998).

Even though **stepwise protocols** increase the ROM of the specimens, the following studies applied a progressive addition of the load:

- **Adding dead weight**: four weights of 25 g, generating a maximum load of 0.3 Nm (Goel et al., 1988).
- **Testing machines** with pneumatic actuators: 1.5 Nm in three increments with 30 s of creep (Panjabi et al., 1988; Panjabi et al., 1991a; Crisco et al., 1991a), 1.0 Nm in three increments with 30 s of creep (Panjabi et al., 2001a), and 3.5 Nm in seven increments with 30 s of creep (Nightingale et al., 2007).

On the other hand, **continuous loading** has been applied in the following studies:

- **Manually loading**, fixing the cervical position with screws (to measure it with CT images): the accurate load is unknown, but it was within the range 100–150 N (Dvorak et al., 1987b).
- **Testing machines** with pneumatic actuators: with a rate of 3°/s (Kettler et al., 2002), or 0.5°/s (Chin et al., 2003), reaching both studies 1.5 Nm.

- **Robotic setup**: with a rate of 10°/s, reaching 1.5 Nm (Tisherman et al., 2020).

Although several studies have applied the same load, **no standardized procedure** has been stated and validated (Zhang et al., 2020). In the experimental tests of this thesis the motion was **continuous and with a rate between 2.5°/s and 4.5°/s**, which is within the range proposed in the literature (0.5°/s–5.0°/s, Wilke et al., 1998a; Wilke et al., 1998b). The rates within the recommended range were practiced by the tester prior to the experimental tests.

In the experimental tests of this thesis, a stepwise loading would have caused a larger ROM (Goertzen et al., 2004); therefore the continuous loading might be more representative of the screening clinical tests in patients, which are conducted with continuous movements. However, previous in vitro studies about the alar ligaments and the cervical kinematics have used stepwise approaches (Panjabi et al., 1991b; Panjabi et al., 1991a), and this should be considered when the kinematics results of these previous studies are compared with the results of this thesis.

To minimize the **viscoelastic effects** in the specimens, the application of three load/unload cycles is well accepted (Crisco et al., 1991a; Wilke et al., 1998a; Panjabi et al., 1988; Panjabi et al., 1991a; Panjabi et al., 2001a; Kettler et al., 2002; Caravaggi et al., 2017; Tisherman et al., 2020). With these previous cycles, the specimens are preconditioned with minimized viscoelastic effects. In the tests of this thesis, these cycles previous to the quantification of the ROM were performed in the three anatomical planes (flexion-extension, lateral bending, and axial rotation; Chapter *Material and Methods*, Section 2.4)

Adding **compressive loads** to C0-T1 specimens during the in vitro mobilizations provide a closer load-displacement response to the in vivo observations (Panjabi et al., 2001b). Compressive loads are sometimes applied in cervical spine specimens (Kettler et al., 2002). But these loads are mainly found in specimens with more vertebral levels than only the upper cervical spine: C0-T1 specimens by Maak et al., 2006, and Panjabi et al., 2001b or C2-T1 by Miura et al., 2002. This is also common in lower spinal segments which include thoracic or lumbar vertebrae (Cripton et al., 2000; Patwardhan et al., 2003; Sis et al., 2016). No compressive load was added in the experimental tests of this thesis, which include only the two uppermost vertebral levels: from C0 to C2.

7.3 Upper Cervical Spine Kinematics

C0-C1 and C1-C2 present unique intervertebral kinematics in comparison with the rest of the cervical spine (Zhou et al., 2020). A relationship between the kinematics of these two uppermost vertebral levels has been reported by an improvement in C1-C2 mobility in the transverse plane after manual therapy in C0-C1 (Hidalgo-García et al., 2016; Malo-Urriés et al., 2017). In most of the cases, the

C0-C1 segment is not included in the studies about intervertebral kinematics (Wu et al., 2007; Reitman et al., 2004; Anderst et al., 2017). The ROM quantification at the craniovertebral joint carried out in this thesis widens the knowledge already available in the literature. A vast number of in vivo and in vitro studies have quantified the motion in lateral bending, axial rotation, flexion, and extension. Some studies have provided intersegmental values (C0-C1 and C1-C2), and others have considered both segments, providing C0-C2 ROM (Table 7.1). Ten in vivo and thirteen in vitro studies are included in this section, showing several methodologies: images such as computed tomography (CT), X-rays, and magnetic resonance imaging (MRI), but also rotatory potentiometers and optical markers tracking. In Table 7.1, one review article is also included: White III and Panjabi, 1978.

7.3.1 Lateral bending

In lateral bending, the lowest ROM to one side has been found in an in vivo study: $1.9 \pm 0.9°$ (C0-C1) and $1.6 \pm 1.3°$ (C1-C2), by Ishii et al., 2006. However, a similar in vivo study, Ishii et al., 2004, which reported axial rotation, has not shown low values in comparison with other studies: their $1.7 \pm 1.5°$ in C0-C1 axial rotation and $36.2 \pm 4.5°$ in C1-C2 are within the range of 1.0°–6.0° for C0-C1 and 23.3°–44.3° for C1-C2 found in the literature (Penning and Wilmink, 1987; Dvorak et al., 1987a; Goel et al., 1988; Panjabi et al., 1991a). This means that the reason for these lower in vitro values in lateral bending might not be their methodology.

About the lateral bending quantified in this thesis, the closest results is from Penning, 1978: 10° considering right and left ROM, and in this thesis the left C0-C2 ROM was $5.58 \pm 3.15°$ and the right ROM was $4.69 \pm 2.30°$, having a total ROM of $10.27 \pm 3.51°$. The largest values have been seen in the in vitro study of Panjabi et al., 1991b: $5.6 \pm 3.0°$ in C0-C1 and $12.6 \pm 7.0°$ in C1-C2 were reported for the left lateral bending, in C0-C3 specimens (Table 7.1). It must be considered that Panjabi et al., 1991b, allowed 30 s of creep after loading steps of 0.5 Nm, and stepwise loading leads to larger ROM (Section 7.2.5, *Loading cervical specimens*).

With the C0-C1 stabilization (Chapter 6), the largest change was in the frontal plane, where the C0-C2 lateral bending was reduced by 55.3%: from $5.6 \pm 3.2°$ to $2.3 \pm 1.2°$ in the left side and from $4.7 \pm 2.3°$ to $2.3 \pm 1.4°$ in the right side. These values of 2.3° are close to the $1.6 \pm 1.3°$ of C1-C2 lateral bending reported by Ishii et al., 2006, but much lower than the rest of the values measured in the rest of the studies of Table 7.1, which are in the range of 4.2°–12.6° (Goel et al., 1988; Panjabi et al., 1988; Panjabi et al., 1991b; Panjabi et al., 2001a; Zhou et al., 2020). This lower value after the C0-C1 stabilization meant even a lower movement at the C1-C2 level, as the screw fixation did not fully restricted the C0-C1 lateral bending. The screw fixation limited the 76.9% of the C0-C1 lateral bending (Chapter 6). In fact, with the intact specimen (Chapter 5), a C1-C2 lateral bending of $2.1 \pm 1.5°$ was quantified to the left and of

1.6 ± 1.2° to the right. These values for the lateral bending show that the C0-C1 stabilization did not clearly limited the C1-C2 in the frontal plane.

7.3.2 Axial rotation

In axial rotation, a higher variability has been observed, but not the same in vitro loads have always been applied. This is the case of Goel et al., 1988, where only 23.3 ± 11.2° were reported in C1-C2, but with a low load of 0.3 Nm in C0-C5 specimens; or the study of Kettler et al., 2002, where 28.5° to the left and 31.6° to the right were measured in C0-C2, but the load was only of 0.5 Nm in C0-C5 specimens. Another example is the study of Panjabi et al., 2001a, with 56.7 ± 4.8° for both sides in C1-C2 axial rotation, which is approximately 28° per side, and the load was larger (1.0 Nm) in C0-C5 specimens. Smaller specimens, from C0 to C3, have been studied with a larger load of 1.5 Nm, quantifying larger C1-C2 axial rotation: 37.4 ± 9.0° to the left and 34.0 ± 9.8° to the right (Panjabi et al., 1991a). A similar ROM (38.9°) was observed with the same load of 1.5 Nm, although the specimens were from C0 to C7 (Panjabi et al., 1988). Among all these studies, Kettler et al., 2002, is the only one with a continuous and constant loading rate (3°/s), the rest of them applied stepwise loading (Goel et al., 1988; Panjabi et al., 2001a; Panjabi et al., 1988; Panjabi et al., 1991a).

The closest results from a previous study to the results of this thesis are the 2.4 ± 1.2° in C0-C1 and 23.3 ± 11.2° in C1-C2, to the left side, by Goel et al., 1988, compared to the measurements of 2.7 ± 2.6° in C0-C1 and 25.3 ± 8.3° in C1-C2 (Chapter 5), also for the left side. However, the results of this thesis were obtained with twice the load: 0.6 ± 0.2 Nm in C0-C2 segments, while Goel et al., 1988, applied 0.3 Nm in C0-C5 segments, and in steps of 0.1 Nm. Other two in vivo studies had similar results to the ROM observed in this thesis: Ishii et al., 2004, with 1.7 ± 1.5° in C0-C1 and 36.2 ± 4.5° in C1-C2, and Salem et al., 2013, with 2.5 ± 1.0° in C0-C1 and 37.5 ± 6.0° in C1-C2.

Two studies from Table 7.1 reported a C0-C1 axial rotation to the opposite side to the head rotation: Penning and Wilmink, 1987, and Iai et al., 1993. Penning and Wilmink, 1987, reported a C0-C1 axial rotation average of 1.0°, in the same direction of the rotation of the head, but up to 2° were quantified to the opposite direction of the head. Iai et al., 1993, showed this "paradoxical counter-rotation" in 30 out of their 40 subjects, with a value of up to 4°. This phenomenon was observed in some of the specimens of this thesis, but in none of them this was observed in left and right axial rotation (Chapter 5).

Related as well with C0-C1 ROM, the rate between C0-C1 and C1-C2 ROM differs among the studies in Table 7.1. Some studies with higher C1-C2 ROM measured very low C0-C1 ROM (1°–2.5°, Penning and Wilmink, 1987; Ishii et al., 2004; Salem et al., 2013). Alternatively, other studies reported higher values for C0-C1 ROM, within the range of 4.4°–6.0°, and with differences in the C1-C2 values of up to 10° (Dvorak et al., 1987b; Panjabi et al., 1991a; Panjabi et al., 2001a). Moreover, the lowest C0-C1 axial rotations were from in vivo studies (Penning and Wilmink, 1987; Ishii et al., 2004; Salem et al., 2013) and the higher values were from in vitro studies (Dvorak et al., 1987b; Panjabi et al., 1991a; Panjabi et al., 2001a). The in vivo analysis of Penning and Wilmink, 1987, actually reported no axial rotation in C0-C1. Only the in vivo study of Dvorak et al., 1987a, showed a value closer to the in vitro studies: 4.2 ± 1.8°.

After C0-C1 stabilization (Chapter 6), the C0-C2 axial rotation to the left side decreased 4.4 ± 3.9°, from 28.0 ± 6.9° to 23.7 ± 8.5°, and to the right side, the ROM decreased 5.4 ± 3.9°, from 33.9 ± 6.7° to 28.5 ± 7.0°. The values after the C0-C1 stabilization are lower than the C1-C2 axial rotations in Table 7.1. Axial rotation was the motion with the highest C0-C1 ROM restriction after the screw fixation, a restriction of 90.9% (Chapter 6). Comparing the C0-C2 axial rotation ROM after the C0-C1 stabilization with the C1-C2 ROM in normal conditions, the C0-C2 axial rotation with the C0-C1 stabilization was slightly lower than the C1-C2 ROM in normal conditions: only 1.6° lower in the left side, from 25.3 ± 8.3° (normal) to 23.7 ± 8.5° (stabilization), and 3.8° lower in the right side, from 32.3 ± 9.3° (normal) to 28.5 ± 7.0° (stabilization). This decreased ROM after the C0-C1 stabilization, even without a full C0-C1 restriction (90.9%), indicates that C0-C1 influenced the C1-C2 axial rotation ROM.

The influence of C0-C1 on C1-C2 observed after the C0-C1 stabilization is in line with the idea that C0-C1 intersegmental motion plays a role in C1-C2 full ROM, presented by Panjabi et al., 1991a, and Ishii et al., 2004. Bogduk and Mercer, 2000, also described that C1-C2 axial rotation is passively influenced by the axial loads in C0-C1 induced by the head. One factor that might have influenced this C1-C2 ROM reduction after C0-C1 stabilization is the alar ligament tightening with the restriction of the C0-C1 movement (Evjenth and Hamberg, 2003). This relationship between these two vertebral levels has been seen in the clinics with mobilizations in C0-C1 which improve C1-C2 ROM (Hidalgo-García et al., 2016; Malo-Urriés et al., 2017).

7.3.3 Flexion and extension

Flexion and extension have always been easier to measure radiologically (in sagittal views) in comparison to C0-C1 rotation (Penning, 1978). But when flexion-extension ROM is quantified in volunteers, attention to the chin position must be taken, as pulling the chin can change ROM measurements (Jones, 1960). Only three in vivo studies with intersegmental values have been found (Lind et al., 1989; Frobin et al., 2002; Zhou et al., 2020). Lind et al., 1989, and Frobin et al., 2002, have provided similar measurements in C0-C1 and C1-C2, while the study of Zhou et al., 2020, has measured half the C0-C1 flexion-extension ROM. These three in vivo studies provide only flexion-extension ROM, without separated flexion and extension. Their values are lower than the reported in this thesis: Lind et al., 1989, measured a C0-C1 flexion-extension ROM of 14 ± 15°, and a C1-C2 ROM of 13 ± 5°, Frobin et al., 2002, reported 14.5 ± 7.65° in C0-C1 and 11.6 ± 4.57° in C1-C2 (males volunteers), and Zhou et al., 2020, measured 6.3 ± 1.6° in C0-C1 and 13.7 ± 4.2° in C1-C2. In the in vitro tests of this thesis, the flexion was 7.2 ± 6.6° in C0-C1 and 12.1 ± 5.8° in C1-C2, and the extension was 11.1 ± 6.4° in C0-C1 and 3.0 ± 2.8° in C1-C2. Among the in vitro studies, it is possible to see separately flexion and extension, but the comparison between the measurements is not possible as Goel et al., 1988, applied 0.3 Nm, Panjabi et al., 2001a, applied 1.0 Nm, and Panjabi et al., 1988, as well as Panjabi et al., 1991b, applied 1.5 Nm, equal to the load in Kettler et al., 2002, but this last study is the only one with continuous loading.

Despite the lower load, Panjabi et al., 2001a, measured with 1.0 Nm a larger flexion (7.2 ± 2.5° in C0-C1 and 12.3 ± 2.0° in C1-C2) than Panjabi et al., 1988, with 1.5 Nm (3.5° in C0-C1 and 11.5° in C1-C2), having both studies C0-C7 specimens. The other study with 1.5 Nm and stepwise loading, Panjabi et al., 1991b, showed twice C0-C1 ROM for flexion: 14.4 ± 3.2°, while equal C1-C2 ROM to the other two studies (12.7 ± 3.2°). In extension, Panjabi et al., 1991b, with 1.5 Nm, showed 14.4 ± 3.2° in C0-C1, a lower ROM than the reported by Panjabi et al., 2001a, with 1.0 Nm: 20.2 ± 4.6°; which apart from having a lower load, this study have more segments, C0-C7, against the C0-C3 specimens in Panjabi et al., 1991b. Similar values were reported in C1-C2 extension ROM by these three studies, within 10.5°–12.1° (Panjabi et al., 1988; Panjabi et al., 1991b; Panjabi et al., 2001a).

With the C0-C1 stabilization (Chapter 6), the flexion-extension ROM was reduced by 46.9%. C0-C2 flexion decreased from 19.8 ± 5.2° to 11.5 ± 4.3°. This change of 8.2 ± 4.1° leaded to the same C1-C2 flexion ROM reported by Panjabi et al., 1988, 11.5°. Nevertheless, the C0-C1 stabilization did not abolish completely the C0-C1 intersegmental motion (Chapter 6). In extension, the reduction in ROM due to the C0-C1 stabilization was similar, 7.7 ± 6.1°, from 14.3 ± 7.7° to 6.6 ± 3.5°. For this movement, Panjabi et al., 1988, reported a larger C1-C2 ROM, 10.9°, but Anderst et al., 2015, reported 8.0 ± 5.5° for C1-C2 extension ROM. Considering the flexion and extension ROM, the average reduction close to 16° is between the range of 13°–19.1° observed in C0-C1 flexion-extension ROM (White III and Panjabi, 1978; Lind et al., 1989; Frobin et al., 2002; Dugailly et al., 2009).

Considering the in vitro values for C1-C2 reported in the tests of this thesis (Chapter 5), C1-C2 showed a flexion of 12.1 ± 5.8° and a extension of 3.0 ± 2.8°. In flexion, the normal C1-C2 flexion (12.1 ± 5.8°) was slightly higher than the value observed after the C0-C1 stabilization (11.5 ± 4.3°), even considering that the stabilization was only partial. This indicated that the screw stabilization of C0-C1 influenced indirectly on the C1-C2 kinematics. However, this effect was not clear for the extension movement. The normal C1-C2 ROM in extension (3.0 ± 2.8°) was half of the C0-C2 ROM observed after the C0-C1 stabilization (6.6 ± 3.5°). As a result, no effect in C1-C2 kinematics after the C0-C1 stabilization was perceived in extension.

7.3.4 Concerns in ROM comparisons

As described in the previous three sections, ROM variability is present in the literature, in both in vivo and in vitro studies (Table 7.1). The variety of experimental methods often complicate the comparison between the kinematics results from different studies (Zhang et al., 2020). This section mentions the mainly concerns related to the comparison between results from different studies:

- **In vitro tests** are common in the literature (Goel et al., 1988; Zhang et al., 2020), but differences when comparing them to **in vivo tests** have been reported, e.g., higher forces are applied in cadavers than when treating patients with manual techniques (Symons et al., 2012), and movements in cadavers can be less accurate due to the removal of soft tissues (Buzzatti et al., 2015) and the lacking of musculature (Bogduk and Mercer, 2000).

- In vitro tests consider **different vertebral levels**, from C0-C3 to C0-C7, or even with the first thoracic vertebra (C0-T1). This is important when tests with same loads but different segments are compared.

- In vitro tests might have **positions which are not natural**: ROM measurements have been done with vertically downwards specimens, therefore without load from the skull to the spinal segments (Dugailly et al., 2009). Furthermore, in vivo tests show **different positions for their participants**: supine or sitting. This might influence the in vivo results, as pelvic incidence correlates with cervical lordosis (Scheer et al., 2013).

- In vivo tests might not represent maximum passive rotation if the **patient is not relaxed**, as muscle spams can restrict the motion and lead to measurement errors (Jones, 1960).

- **The methodology to measure**, e.g., medical images, optoelectronic systems, rotatory potentiometers, etc., and its accuracy in quantifying ROM (Section 7.2.4, *Motion quantification*).

- In vitro tests show **different loads and different methods to apply them**: continuous or stepwise, and mechanical or manual. The protocol to load the specimen influences ROM (Section 7.2.5, *Loading cervical specimens*). Some of the loads in previous studies might not have reached the maximum ROM, as Goel et al., 1988, with only 0.3 Nm. There are in vitro studies which did not aim for maximum ROM, but for the relationship between ROM and different applied loads (Goel et al., 1988; Panjabi et al., 1988).

- Studies can have different **age** groups, which might influence the result comparison. The effect that age has on the upper cervical spine remains uncertain in the literature. Decreased neck ROM with age has been reported (Lind et al., 1989). And a decreased upper cervical spine ROM with the flexion-rotation test has also been observed in healthy subjects (Schäfer et al., 2020). By contrast, also with the flexion-rotation test, no age effect on the mobility has been found (Smith et al., 2008), and no alterations in C0-C2 ROM between young (20–29 years) and middle-aged adults (50–59 years) have been seen in radiographs (Park et al., 2014). Furthermore, Castro et al., 2000, observed that the C0-C2 axial rotation ROM can increase with age to compensate the decrease of motion in the lower cervical levels.
- **The morphological variability** plays also a role in ROM quantification, but the consideration of this aspect is more complex. For example, different orientations for the alar ligaments have been reported (caudocranial, horizontal, and craniocaudal; Section 1.3.2, *Alar ligament orientation*), which could influence intersegmental kinematics, but no study has considered these anatomical differences in the C0-C2 kinematics. Differences in the bones dimensions could also influence the kinematics (Stemper et al., 2011; John et al., 2019).

Study	Method	n	Lat. Bending	Axial Rotation	Flexion	Extension	Comments
Penning, 1978	vv (Xr)	20	L+R: 10	L+R: 0 + 70	30 (25–45) + 30 (25–45)		
White III and Panjabi, 1978	Review	-	L+R: 8 (4–14) + 0 (0)	L+R: 0 (0) + 47 (22–58)	13 (4–33) + 10 (2–21)		
Penning and Wilmink, 1987	vv (CT)	26		L=R: 1.0 (-2–5) + 40.5 (29–46)			Supine position
Dvorak et al., 1987b	vt (CT)	12		L: 5.9±3.6 + 33.0±5.4			100–150 N, full-ROM
	C0-C3			R: 4.4±2.6 + 31.4±9.7			Manual load
alar ligament cut				L: 7.9±3.9 + 33.0±5.1			
left side				R: 9.4±2.3 + 37.2±8.8			
Dvorak et al., 1987a	vv (CT)	9		L: 3.8±1.5 + 44.3±5.2			Supine position
				R: 4.2±1.8 + 41.8±5.7			
Goel et al., 1988	vt (m) *C0-C5*	8	L: 3.4±2.8 + 4.2±2.8	L: 2.4±1.2 + 23.3±11.2	6.5±2.5 + 4.9±2.0	16.5±7.6 + 5.2±2.9	0.3 Nm (steps: 0.1 Nm)
Panjabi et al., 1988	vt (m) *C0-C7*	10	L=R: 5.5 + 6.7	L=R: 7.2 + 38.9	3.5 + 11.5	21.0 + 10.9	1.5 Nm (3 steps)
Lind et al., 1989	vv (Xr)	70			14±15 (2–30) + 13±5 (2–28)		Sitting (back support)
Panjabi et al., 1991b	vt (m)	10	L:5.6±3.0 + 12.6±7.0	L: 3.3±2.3 + 37.4±9.0	14.4±3.2 + 12.7±3.2	14.4±3.2 + 10.5±5.0	1.5 Nm (3 steps)
Panjabi et al., 1991a	*C0-C3*		R: 5.1±2.5 + 8.3±4.5	R: 6.0±4.5 + 34.0±9.8			30 s creep
alar ligament cut			L: 5.4±2.8 + 13.5±6.5	L: 5.0±2.7 + 39.0±9.5 (1.5 Nm)	16.3±3.7 + 16.2±3.7	15.3±4.9 + 11.8±3.7	
left side			R:6.1±2.3 + 9.6±4.5	R: 8.1±2.9 + 36.1±10.4 (1.5 Nm)			
Panjabi et al., 2001a	vt (m) *C0-C7*	8	L+R = 9.1±1.5 + 6.5±2.3	L+R = 9.9±3.0 + 56.7±4.8	7.2±2.5 + 12.3±2.0	20.2±4.6 + 12.1±6.5	1.0 Nm (3 steps, 30 s)
Frobin et al., 2002	vv (Xr)	18-61			14.5±7.65 + 11.6±4.57 (males); 12.6±6.83 + 10.9±4.87 (fem)		
Kettler et al., 2002	vt (p)	6	L: 7.5 (1.5 Nm)	L: 28.5 (0.5 Nm)	17.5 (1.5 Nm)	17.4 (1.5 Nm)	Constant load
	C0-C5		R: 8.0	R: 31.6			(3°/s)
alar ligament cut			L: 9.7	L: 32.4	19.2	18.6	
left side			R: 10.1	R: 34.6			
Chin et al., 2003	vt (m) *C0-C3*	4	L+R: 9.1 + 8.1		19.3 + 13.1		1.5 Nm (0.5°/s)
Ishii et al., 2004; Ishii et al., 2006	vv (MRI)	15; 12	L=R: 1.9±0.9 + 1.6±1.3	L=R: 1.7±1.5 + 36.2±4.5			
Nightingale et al., 2007	vt (m) *C0-C3*	16			51.4±9.3		3.5 Nm (steps: 0.5 Nm)
Cattrysse et al., 2007a	vt (e)	6			16 + 14±4		Manual load
Cattrysse et al., 2007b	C0-T1	6	2.88±2.39 + 7.10±4.78	3.88±2.03 + 49.94±5.52			
Dugailly et al., 2009	vt (2) *C0-C3*	10		L=R: 5.0±3.4 + 46.1±12.5	19.1±5.8 + 14.3±3.3		Load-step, downwards
Salem et al., 2013	vv (CT)	20		L=R: 2.5±1.0 + 37.5±6.0			Passive Rot; Supine
Anderst et al., 2015	vv (Xr)	29			C1-C2: L=R: 7.7±3.8	C1-C2: 8.0±5.5	Sitting
Anderst et al., 2017	vv (Xr)	20		C1-C2: 37.8±8.6 (22.4–55.5)			Sitting
Zhou et al., 2020	vv (f)	8	L+R: 1.9±1.5 + 7.6±2.7	L+R: 3.7±2.2 + 72.9±7.6	6.3±1.6 + 13.7±4.2		Sitting
Lorente et al., 2021	vt (m)	10	L: 3.3±1.6 + 2.1±1.5	L: 2.7±2.6 + 25.3±8.3	7.2±6.6 + 12.1±5.8	11.1±6.4 + 3.0±2.8	Manual load
Chapter 5	*C0-C2*		R: 3.1±2.2 + 1.6±1.2	R: 1.2±3.5 + 32.3±9.3			Constant load
alar ligament cut			L: 4.1±1.3 + 3.3±2.3	L: 3.8±6.0 + 27.2±10.7	6.1±4.7 + 16.2±6.0	13.7±4.9 + 2.6±2.3	
right side			R: 4.1±2.1 + 1.8±1.1	R: 3.2±3.9 + 32.4±9.4			

vv = in vivo; vt = in vitro; m = markers tracking; Xr: X-rays; p: rotatory potentiometer; e: electromagnetic device; mCT: markers digitizing + models from CT; fCT: fluoroscopy + models from CT
values in brackets: minimum - maximum

TABLE 7.1: Range of motion in previous studies: lateral bending, axial rotation, flexion, and extension.

7.4 Clinical Implications

The screening of the upper cervical stability is important prior to manual interventions in these cervical levels to avoid devastating neurovascular consequences (Mintken et al., 2008; Rushton et al., 2014). The tests of this thesis simulated, with an in vitro setup, two clinical techniques which assess the cervical spine stability: the side-bending stress test (Aspinall, 1990) and the rotation stress test (Mintken et al., 2008). The manual mobilizations for these two tests were performed by the same practitioner with more than 15 years of experience in manual therapy. The applied manual techniques are to assess the upper cervical spine instability by evaluating the integrity of the alar ligaments (Section 1.4.2, *Manual therapy*).

The clinical validity and reliability of the side-bending stress test and the rotation stress test need to be further investigated (Kaale et al., 2008; Osmotherly et al., 2012; Hutting et al., 2013; Von et al., 2018). Moreover, their safety has been questioned under certain scenarios (Hufnagel et al., 1999; Fabio, 1999). More biomechanical studies simulating manual clinical tests would provide a better understanding about what clinicians can expect while assessing a patient.

In the alar ligaments, previous studies have identified an unilateral response during the lateral bending and the axial rotation movements: only the contralateral alar ligament increased its length (Dvorak et al., 1987b; Panjabi et al., 1991b; Osmotherly et al., 2012; Zhou et al., 2021). By contrast, Panjabi et al., 1991a, and Kettler et al., 2002, reported a bilateral increase after evaluating the effects of an unilateral transection of the alar ligaments.

This section presents, from a clinical point of view, the relevance of the bilateral effects observed in the in vitro tests of this thesis after unilateral alar ligament transection (Section 7.4.1), as well as the effects of the alar ligament transection on the C0-C2 stiffness (Section 7.4.2). The in vitro results observed after the C0-C1 stabilization are also applicable in the clinics (Section 7.4.3).

7.4.1 Stress tests: side-bending and rotation

One statement used to describe the **side-bending stress test** is that no motion between C0 and C2 is expected in the frontal plane when the alar ligaments are intact (Osmotherly et al., 2012). Furthermore, lateral bending at C1-C2 has been reported as nonexistent (Penning and Wilmink, 1987; White III and Panjabi, 1978). However, lateral bending was observed at C0-C1 and C1-C2 in the in vitro tests of this thesis, as in other biomechanical studies (Section 7.3.1, *Lateral bending*). The fact that lateral bending does not occur at the upper cervical spine should be revised as this motion might be observed in healthy patients during the side-bending stress tests.

The intersegmental kinematics provided more detailed information about the right and left sides: after the unilateral alar ligament transection in the right side, both sides increased their ROM but the increase in the C1-C2 level was 1.2 ± 1.3° to the left side, while to the right side it was only 0.2 ± 0.8°. On the other hand, the ROM increase in the C0-C1 level was much more similar between the left side (0.8 ± 1.1°) and the right side (1.0 ± 1.2°) after the alar ligament transection. The knowledge about these differences between the left and right sides, at the intersegmental level, has provided new information in the literature related to the alar ligament assessment (Chapter 5).

The side-bending stress test has a lower effect on the contralateral alar ligament than the **rotation stress test** (Osmotherly et al., 2012). In the rotation stress test, the C0-C2 ROM is evaluated but different limits for a positive test have been reported. Osmotherly et al., 2013a, measured in vivo C0-C2 axial rotation and reached the conclusion that intact alar ligaments would show a cut-off value of 21° or less; showing with their participants an average ROM of 10.6°. The cut-off value of 20° has been considered in other studies (Von et al., 2018). However, values of up to 40° have been considered to represent intact alar ligaments (Hing and Reid, 2004). In the tests of this thesis, as well as in other in vitro and in vivo studies, greater average values than 10.5° have been reported, reaching even more than the double of C0-C2 axial rotation (Section 7.3.2, *Axial rotation*). Although the rigid attachment of C2 in the in vitro tests of this thesis must be considered when the kinematics results are compared with other studies, even if the other in vivo studies have the C2 level stabilized: the in vivo stabilizations of vertebral levels can be less restrictive (Gianola et al., 2015). Larger values than 10.5° might be expected in healthy population during the rotation stress test. Furthermore, the larger values found in the literature mean that the limit around 20° for the rotation stress test might not cover its full ROM. Despite this disagreement in the ROM limit, a good reliability has been described for the rotation stress test in comparison with MRI findings (Kaale et al., 2008).

In axial rotation, at the intersegmental level after the unilateral alar ligament transection in the right side, no effect was observed for the right ROM at the C1-C2 level: the increase was from 32.3 ± 9.3° to 32.4 ± 9.4°. The left ROM increased at the C1-C2 level from 25.3 ± 8.3° to 27.2 ± 10.7°. In contrast to these changes: the effects on the C0-C1 segment were slightly more marked to the right side: from 1.2 ± 3.5° to 3.2 ± 3.9°, while the change in the left side was from 2.7 ± 2.6° to 3.8 ± 6.0° (Chapter 5).

These manual tests have been shown as helpful clinical tools in the examination of the upper cervical instability (Von et al., 2018). Nevertheless, **caution when extrapolating the work of this thesis to the clinics** must be taken. Only a moderate inter-examiner agreement of 87% has been reported in the upper cervical spine mobility assessment by physical therapists (Smedmark et al., 2000). In a patient with an alar ligament injury, whether the clinicians can detect the slightly differences in the ROM and stiffness herein reported after the alar ligament transection is unclear. As previously stated by Osmotherly et al., 2012, these standard clinical stress tests might not be accurate for some patients due to anatomical variations.

Although in this thesis the **motion in the sagittal plane** has not been clinically evaluated, flexion-extension was influenced by the alar ligament transection as well. In this plane, an increase of the intersegmental ROM after the alar ligament transection was not always detected: C0-C1 flexion and C1-C2 extension decreased (C0-C1 flexion: from 7.2 ± 6.6° to 6.1 ± 4.7°; C1-C2 extension: from 3.0 ± 2.8° to 2.6 ± 2.3°; Chapter 5).

Manual mobilizations in the sagittal plane are common (Cattrysse et al., 2007a), and sometimes are combined with movements in other anatomical planes, e.g., flexion-rotation test (Ogince et al., 2007; Hall et al., 2010; Dunning et al., 2012). Previous studies have suggested that the assessment of the upper cervical spine stability has to be performed in the planes of the neutral position, flexion, and extension, to consider the variability of the orientation in the alar ligaments (Aspinall, 1990). A similar idea has been suggested more recently: a combination of these manual tests and others, e.g., lateral shear test or flexion-rotation test, may help in determining the assessment of the alar ligaments (Von et al., 2018). Applying movements in more than one anatomical plane at the same time can help in the detection of instabilities of the upper cervical spine (Lorente et al., 2022). However, when the motion is assessed in different anatomical planes one after the other, no protocol about the sequence for the anatomical planes exists (Mintken et al., 2008).

7.4.2 Stiffness against maximum mobility

In **lateral bending**, after the alar ligament transection, a bilateral effect was observed in the in vitro tests of this thesis: the ROM increased from 4.69 ± 2.30° to 5.99 ± 2.48° in the right side and from 5.58 ± 3.15° to 7.46 ± 3.55° in the left side (Chapter 3). These increases of 1.30 ± 1.54° in the right side and of 1.88 ± 1.51° in the left side (Table 7.1) might be hardly detected by a clinician. However, a **decrease in the stiffness** of C0-C2 after the alar ligament transection has also been quantified when comparing the lateral bending reached with 2 N, 4 N, and 6 N (0.26 Nm, 0.52 Nm, and 0.78 Nm). In these three load values, the right and left lateral bending increased after the unilateral alar ligament transection, being statistical significant with the load of 6 N (Chapter 3). With the maximum applied load, in the right side, the larger ROM after the alar ligament transection was reached with a lower maximum load than before the transection: 6.44 ± 4.89 N were applied before the transection, whereas after the transection (with the increase of 1.30 ± 1.54°), a load of 6.07 ± 1.82 N was measured. This decrease in the maximum load was not observed in the left side: the load increased 1.67 ± 3.30 N after the alar ligament transection.

In **axial rotation**, after the alar ligament transection, a bilateral effect occurred: the ROM increased from 33.9 ± 6.6° to 38.5 ± 9.5° in the right side and from 28.0 ± 6.9° to 31.6 ± 6.5° in the left side (Chapter 4). As described in the previous section for the stiffness in the lateral bending motion: the stiffness of C0-C2 also decreased after the alar ligament transection. In this case, the axial rotation was quantified in two values before reaching the maximum ROM: 1 N and 2 N (0.15 Nm and 0.30 Nm). For these two loads, in both sides, the rotation increased after the unilateral alar ligament transection. However, only in the right side, these differences between before and after the ligament transection were statistically significant.

The neutral zone is defined as the range from the neutral position to the position which can be reached under zero load. When loading specimens, the neutral zone has been found to be more sensitive to injuries than the range of motion (Oxland and Panjabi, 1992). In the simulation of both tests, side-bending and rotation stress tests, the stiffness of the specimens decreased after the alar ligament transection. This relationship between the induced injury and the C0-C2 stiffness supports that considering the **resistance perceived by the clinicians** during the side-bending and rotation stress tests might have a role in the assessment of the upper cervical spine stability. Another reason to consider the change in the stiffness is that the upper cervical spine show a wide range of rotational mobility in a healthy population (Lummel et al., 2012). Therefore, the change in the stiffness quantified after the alar ligament transection could be a relevant indicator associated to the ROM variation.

7.4.3 Indirect mobilizations on C0-C1

The hypomobility in the C0-C1 segment might decrease the C1-C2 mobility due to the tightening of the alar ligaments (Hidalgo-García et al., 2016). Previous studies with patients have reported an improvement in the C1-C2 mobility after mobilizing the C0-C1 segment (Hidalgo-García et al., 2016; Malo-Urriés et al., 2017; González-Rueda et al., 2020). This is highly important when the mobilizations concern the upper cervical spine: treating other cervical level would minimize the risk related to neurovascular consequences (Fabio, 1999; Hufnagel et al., 1999). The risk of these manual techniques in the upper cervical spine has been extensively studied in the literature, e.g., cases of arterial dissection and lesions of the brain stem; caution must be present due to the proximity of the cervical arterial system (Fabio, 1999; Hufnagel et al., 1999).

A guide about the best practices in the assessment of the cervical spine has been published (Rushton et al., 2014). This guide supports the emerging manual techniques with indirect treatments applied in a different spine level to where the pain is located (Rushton et al., 2020). However, no biomechanical study has previously analyzed the relationship between the C0-C1 and C1-C2 kinematics after a restriction of the C0-C1 mobility. This analysis is needed to better understand the relationship between these two cervical levels and support the relationship between the C0-C1 and C1-C2 mobility observed in the clinics (Chapter 6). After the C0-C1 stabilization, reductions in C0-C2 ROM were detected in the three anatomical planes (55.3% in the frontal plane, 46.9% in the sagittal plane, and 15.6% in the transverse plane), and the restriction of the C0-C1 mobility was not fully limited (restrictions of 76.9% in the frontal plane, 74.4% in the sagittal plane, and 90.9% in the transverse plane) by the screw stabilization (Section 2.2, *Anatomical Procedures*).

Furthermore, the analysis herein performed about the influence of the C0-C1 hypomobility on the ROM of the upper cervical spine can be of interest to evaluate different atlanto-occipital screw fixation (Xu et al., 2019).

7.5 Strengths and Limitations

The results presented in this thesis should be considered after understanding the following strengths and limitations:

7.5.1 Strengths

The experimental tests of this thesis have the following strengths in comparison with previous studies:

- The vertebra C2 was fixed to simulate the clinical screening tests which manually produce **C2 stabilization** (Osmotherly et al., 2013a). Although previous in vitro studies have quantified the upper cervical spine ROM before and after alar ligament transection in flexion-extension (Panjabi et al., 1991b; Kettler et al., 2002; Tisherman et al., 2020), lateral bending (Panjabi et al., 1991b; Kettler et al., 2002; Tisherman et al., 2020), and axial rotation (Dvorak et al., 1987b; Panjabi et al., 1991a; Kettler et al., 2002; Tisherman et al., 2020), this thesis presents the first in vitro testing with alar ligament transection and C2 fixed.

- The results presented in this thesis provide **continuous data about the kinematic response within the full ROM** in flexion-extension, lateral bending, and axial rotation. A stepwise loading of the specimens would have caused a larger ROM (Goertzen et al., 2004; Section 7.2.5, *Loading cervical specimens*).

- The specimens were **manually mobilized** in the experimental tests of this thesis. Loading manually the specimens provided to the physiotherapist a real-feel of the behavior of each specimen. The previous studies related to the upper cervical spine kinematics after alar ligament transection were conducted using testing machines (Panjabi et al., 1991b; Panjabi et al., 1991a; Kettler et al., 2002; Tisherman et al., 2020). Only Dvorak et al., 1987b, moved the specimens manually, but the load was not specified, mentioning a wide range (100–150 N) to reach the axial rotation full-ROM.

- A consistent neutral reference for the initial position was defined in the experimental protocol, and two red-light lasers assured the neutral orientation in the sagittal and transverse planes using anatomical references of the skull (Section 2.3, *Biomechanical Procedures*). No anatomical references related to the neutral position have been given in other in vitro studies (Dvorak et al., 1987b; Panjabi et al., 1988; Section 7.2.3, *Initial positioning of the specimens*).

The C2 fixation, the continuous load, and the manual mobilization of the specimens have contributed to present in this thesis the first biomechanical studies which simulate clinical tests (side-bending stress test and rotation stress test) before and after alar ligament transection.

7.5.2 Limitations

The following limitations need to be considered:

- The **sample size** consisted of only ten specimens. A sample like this cannot truly represent anatomical variability and normal kinematics of the population. Variability in cervical spine kinematics has been considered as normal (Christensen and Nilsson, 1998); and enormous ranges have been described when presenting normal ROM in healthy individuals (Bogduk and Mercer, 2000). Furthermore, previous studies have shown a high variability in the morphology of the alar ligaments (Cattrysse et al., 2007c; Lummel et al., 2010), which could influence the kinematic response of the ten specimens after the unilateral alar ligament transection.

- The ten specimens of these tests cannot be equally divided into two groups by **gender**: there were nine male specimens and only one female specimen. This could have influenced the measured ROM, as women have shown larger ROM and lower neck strength than men in previous studies (Ferrario et al., 2002; Matsunaga et al., 2001; Nightingale et al., 2007). However, other studies have not seen this influence by gender (Lind et al., 1989; Trott et al., 1996). Furthermore, the study of Dvorak et al., 1992, has found no significant differences in neck kinematics between men and women, in subjects over 60 years old, and the ten specimens of this thesis were over that age, in the 63–85 year range.

- The advanced **age** of the ten specimens (74 years, range: 63–85) might have influenced the ROM presented in the results. Cervical spine ROM has shown a decrease with age (Matsunaga et al., 2001), but Park et al., 2014, showed that a group of asymptomatic adults in their 50s did not show any age influence in comparison to a group in their 20s in C0-C2 flexion-extension ROM. C0-C2 axial rotation might actually increase with age to compensate the decreased motion in the lower cervical segments (Castro et al., 2000). The preservation of C0-C2 motion might be influenced by the lack of intervertebral discs within these vertebral levels (Park et al., 2014).

- The **intact anatomy** of all the structures could not be kept. The important aspect is that no ligament was affected in the preparation of the specimens, apart from the unilateral transection of the alar ligament to study its effects on kinematics. However, other anatomical structures were carefully removed to expose the right side of the alar ligament. These affected structures were the brainstem, spinal cord, dura, and part of the tectorial membrane.

- Similar to the previous point, there is also another aspect related to anatomical variations when comparing the in vitro tests to patients with alar ligament injuries. **Alar ligament injuries** with a complete rupture of the ligament, as simulated in this thesis, can show a bone fracture (Bloom et al., 1996) or the deviation of the dens toward C1 (Unal et al., 2019). No bone fractures were replicated in the in vitro tests of this thesis. Therefore, the cervical instability in the in vitro tests cannot be directly comparable to all clinical scenarios.

- The **flexion** restraint might differ between these in vitro conditions and in vivo conditions: the posterior neck muscles play a role in limiting flexion, as well as the restriction by the contact of the atlantial sockets against the base of the skull (Goel et al., 1988; Bogduk and Mercer, 2000). This second restraint system (bone-to-bone contact) might have played the main role in the flexion ROM of the in vitro tests, due to the lack of neck musculature in the specimens.

- In order to transfer the results of this thesis into the **clinics**, it must be considered that the in vitro ROM alterations measured might not be always detected by practitioners in clinical assessments. The maximum ROM increase was 4.4° in lateral bending (Chapter 3) and 12.0° in axial rotation (Chapter 4).

- Another consideration related to the clinics is that not all the specimens that were tested showed a **larger ROM after the unilateral alar ligament transection**. Few exceptions were seen, therefore, the common response observed in the in vitro tests could not be the response that a patient with an alar ligament injury shows in manual examination.

Absence of muscle tone might be a consideration in some biomechanical studies. During the preparation of the C0-C2 specimens, muscle tissue was removed to track the kinematics of the vertebrae and visualize the right alar ligament. Nevertheless, artificial muscles have been rarely used in previous studies and this experimental setup without simulated muscle forces to study spinal instability can be used when the results are compared to the own initial intact state (Kettler et al., 2002).

7.6 Scientific Dissemination

During the development of this thesis, the following publications in peer-reviewed journals and presentations in conferences have been accomplished.

7.6.1 Peer-reviewed journals

1. Hidalgo-García, C., Lorente, A.I., Rodríguez-Sanz, J., Tricás-Moreno, J.M., Simon, M., Maza-Frechín, M., Lopez-de-Celis, C., Krauss, J. and Pérez-Bellmunt, A., 2020. Effect of Alar Ligament Transection in Side-bending Stress Test: A Cadaveric Study. *Musculoskeletal Science and Practice*, 46, p.102110.
   https://doi.org/10.1016/j.msksp.2020.102110
   Impact Factor: 2.520; JCR Quartile: Q2, Rehabilitation

2. Hidalgo-García, C., Lorente, A. I., Lucha-López, O., Auría-Apilluelo, J. M., Malo-Urriés, M., Rodríguez-Sanz, J., López-de-Celis, C., Maza-Frechín, M., Krauss, J. and Pérez-Bellmunt, A., 2020. The Effect of Alar Ligament Transection on the Rotation Stress Test: A Cadaveric Study. *Clinical Biomechanics*, 80, p.105185.
   https://doi.org/10.1016/j.clinbiomech.2020.105185
   Impact Factor: 2.063; JCR Quartile: Q3, Biomedical Engineering

3. Lorente, A.I., Hidalgo-García, C., Rodríguez-Sanz, J., Maza-Frechín, M., Lopez-de-Celis, C. and Pérez-Bellmunt, A., 2021. Intersegmental Kinematics of the Upper Cervical Spine: Normal Range of Motion and its Alteration After Alar Ligament Transection. *Spine*, 46 (24), p.E1320.
   https://doi.org/10.1097/BRS.0000000000004167
   Impact Factor (2020): 3.468; JCR Quartile (2020): Q1, Orthopedics

4. Hidalgo-García, C., Lorente, A.I., López-de-Celis, C., Lucha-López, O., Malo-Urriés, M., Rodríguez-Sanz, J., Maza-Frechín, M., Tricás-Moreno, J.M., Krauss, J. and Pérez-Bellmunt, A., 2021. Effects of Occipital-atlas Stabilization in the Upper Cervical Spine Kinematics: an in Vitro Study. *Scientific Reports*, 11(1), p.1-13. https://doi.org/10.1038/s41598-021-90052-6
   Impact Factor (2020): 4.380; JCR Quartile (2020): Q1, Multidisciplinary Sciences

5. Lorente, A.I., Hidalgo-García, C., Fanlo-Mazas, P., Rodríguez-Sanz, J., López-de-Celis, C., Krauss, J., Maza-Frechín, M., Tricás-Moreno, J.M. and Pérez-Bellmunt, A., 2022. In Vitro Upper Cervical Spine Kinematics: Rotation with Combined Movements and its Variation after Alar Ligament Transection. *Journal of Biomechanics*, 130, p.110872.

https://doi.org/10.1016/j.jbiomech.2021.110872
Impact Factor (2020): 2.712, JCR Quartile (2020): Q3, Biomedical Engineering

7.6.2 Conferences

International conferences

1. Lorente, A.I., Hidalgo-García, C., Rodríguez-Sanz, J., Maza-Frechín, M., Pérez-Bellmunt, A. Upper Cervical Spine Rotation After Occipital-Atlas Stabilization: Rotation with Flexion and Ipsilateral Lateral Bending. *ORS Annual Meeting (Orthopaedic Research Society)*. Virtual. 12-16 February 2021.

2. Hidalgo-García, C., Lorente, A.I., Tricás-Moreno, J.M., Maza-Frechín, M., Pérez-Bellmunt, A., Rodríguez-Sanz, J. Upper Cervical Biomechanics: Research Informing Practice. *KEOMT 2019 Global Conference*, Rochester (MI, USA). June 7-9, 2019.

Other conferences

1. Lorente, A.I., Hidalgo-García, C., Rodríguez-Sanz, J., Maza-Frechín, M., Pérez-Bellmunt, A. Upper Cervical Spine Instability: In Vitro Simulation of Manual Therapy Techniques. *I Annual Conference of Doctorate Students, Universidad Miguel Hernández*. Virtual. February 2, 2021.

2. Lorente, A.I., Hidalgo-García, C., Rodríguez-Sanz, J., Maza-Frechín, M., Pérez-Bellmunt, A. Range of Motion of the Upper Cervical Spine: Flexion, Extension, Lateral Bending, and Axial Rotation. *IX Conference for Young Researchers of the I3A Research Institute*, ISSN 2341-4790, vol. 8. Virtual. December 11, 2020.
https://papiro.unizar.es/ojs/index.php/jji3a/article/view/4877/3999

3. Lorente, A.I., Maza Frechín, M., Pérez Bellmunt, A., Hidalgo García, C. Upper Cervical Spine Stability: Maximum Rotation and the Rotation Stress Test in Clinics. *VIII Conference for Young Researchers of the I3A Research Institute*, ISSN 2341-4790, vol. 7. Zaragoza (Spain). June 6, 2019.
https://papiro.unizar.es/ojs/index.php/jji3a/article/view/3537/3094

4. Hidalgo García, C., Tricás Moreno, J.M., Maza Frechin, M., Lorente, A.I., et al. Efecto de la sección transversal del ligamento alar en el rango de movimiento de la inclinación y rotación cervical superior. *3rd OMT-E Congress*, Zaragoza (Spain). June 8-9, 2018.

5. Lorente, A.I., Hidalgo García, C., Frechín Maza, M., et al. Estudio in vitro del efecto de movimiento acoplado y no acoplado en la rotación cervical superior. *II Congreso Internacional en Ciencias de la Salud y del Deporte*, Huesca (Spain). May 23-25, 2019.

6. Hidalgo García C, Lorente AI, Maza Frechín M, et al. Estudio in vitro de la fijación del segmento occipital-atlas en la inclinación cervical superior. *II Congreso Internacional en Ciencias de la Salud y del Deporte*, Huesca (Spain). 23-25 May 2019.

7.6.3 Dissemination in other research lines

Considering only the time period of this thesis, the following contributions have been accomplished in other research areas:

1. Juste-Lorente, Ó., Maza, M., Piqueras, A., Lorente, A.I., López-Valdés, F.J., 2022. Effects of including a penetration test in motorcyclist helmet standards: influence on helmet stiffness and impact performance. *Applied Sciences*, 12(5), p.2455.
   https://doi.org/10.3390/app12052455
   Impact Factor (2020): 2.679; JCR Quartile (2020): Q2, Engineering, Multidisciplinary

2. Rodríguez-Sanz, J., Malo-Urriés, M., Corral-de-Toro, J., López-de-Celis, C., Lucha-López, M.O., Tricás-Moreno, J.M., Lorente, A.I., Hidalgo-García, C.,2020. Does the Addition of Manual Therapy Approach to a Cervical Exercise Program Improve Clinical Outcomes for Patients with Chronic Neck Pain in Short-and Mid-Term? A Randomized Controlled Trial. *International Journal of Environmental Research and Public Health*, 17(18): 6601. https://doi.org/10.3390/ijerph17186601
   Impact Factor: 3.390; JCR Quartile: Q2, Public, Environmental & Occupational Health

3. Juste-Lorente, Ó., Maza-Frechin, M., Lorente, A.I., Lopez-Valdes, F., 2018. Differences in the kinematics of booster-seated pediatric occupants using two different car seats. *Traffic Injury Prevention*, 19(1): 18-22.
   https://doi.org/10.1080/15389588.2017.1334119
   Impact Factor: 1.465; JCR Quartile: Q3, Environmental & Ocupational Health

4. Piqueras-Lorente, A., Iraeus, J., Lorente, A.I., et al. Kinematic assessment of subject personification of human body models (THUMS). *IRCOBI Conference (International Research Council on the Biomechanics of Injury)*, Athens (Greece). September 12-14, 2018.

5. Lorente, A.I., Maza, M., Juste, O., Piqueras, A., López-Valdés, F.J. Thoracic Deformation in Nearside Oblique Sled Impacts. *Virtual Physiological Human Conference (VPH2018)*, Zaragoza (Spain). September 5-7, 2018.

6. Piqueras, A., Juste-Lorente, O., Lorente, A.I., Maza, M. Influence of the head size in the injury metrics for oblique impacts. *Virtual Physiological Human Conference (VPH2018)*, Zaragoza (Spain). September 5-7, 2018.

7. Lopez-Valdes, F., Juste, O., Lorente, A., et al. Comparison of the Kinematics and Dynamics of the THOR-50M Dummy and Elderly Volunteers in Low-Speed Frontal Decelerations. *IRCOBI conference (International Research Council on the Biomechanics of Injury)*, Antwerp (Belgium). September 13-15, 2017.

8. Lopez-Valdes, F.J., Juste-Lorente, O., Lorente, A., et al. Kinematics and dynamics of young and elderly occupants in low speed frontal tests. *61st Annual Scientific Conference of the Association for the Advancement of Automotive Medicine (AAAM)*, Las Vegas (USA). October 15-18, 2017.

7.7 Future Work

The protocol of the experimental tests of this thesis was designed to measure the **elongation of the alar ligaments** during all the manual mobilizations. However, the quantification of these elongations was not included in the objectives of the thesis and these data have not been analyzed yet. In the future, the research team aims to publish how the elongation of both alar ligaments varied in all the manual mobilizations conducted, i.e., mobility in the three anatomical planes, C0-C1 stabilization, and the intact side after the unilateral alar ligament transection. The elongation of the alar ligaments has been previously reported during axial rotation and lateral bending in healthy volunteers using MRI (Osmotherly et al., 2012) or fluoroscopy (Zhou et al., 2021).

Furthermore, in the same research line as this thesis, the research team is starting a new in vitro study which considers **specimens from the head to the first thoracic vertebra** (head-T1). In the tests protocol, T1 is rigidly attached to a load cell and the head is manually moved with the same metallic handlebar used in the test of this thesis (Chapter 2, *Material and Methods*).

In this new study, instead of stabilizing C0-C1, **a surgical protocol is replicated to fix C2-C3**. With C2-C3 fixed, the goal is to quantify how the restriction of the C2-C3 mobility might reduce the upper cervical spine ROM due to the tightening of the alar ligaments, reducing as well the head-T1 ROM.

7.8 Conclusions

7.8.1 Conclusions

The in vitro tests presented in this thesis, with head-C2 specimens, have provided the literature with continuous measurements of the full range of motion (ROM) in the three anatomical planes (lateral bending, axial rotation, flexion-extension), with the end-feel by a physical therapist. Apart from analyzing the intersegmental kinematics, **C0-C1 was stabilized** with screws to quantify its effect on the segment immediately below it (C1-C2). After removing the C0-C1 stabilization, the measurements were repeated in the three anatomical planes with a **unilateral alar ligament transection** to prove the influence of this ligament on the upper cervical spine kinematics. Widening the knowledge of all these aspects related to the upper cervical spine biomechanics is highly interesting from a clinical point of view. The practitioners conducting manual therapy techniques to assess the upper cervical spine stability need to have further scientific evidence concerning the intersegmental response under manual loading and the ways in which an alar ligament injury or a C0-C1 hypomobility disrupt this intersegmental behavior. These topics were addressed in the four research articles of this thesis.

In vivo results are more attractive to clinicians; a direct comparison between in vitro and in vivo results is difficult. Some aspects might reduce the **biofidelity** of the in vitro conditions, e.g., the removal of soft tissues and the absence of body reactions. However, the use of cadavers in biomechanics is widely accepted and allows the replication of alar ligament injuries by cutting one side of this bilateral ligament and comparing in the same specimen the alterations that this unilateral transection causes in mobility. Furthermore, other considerations were included in the test protocol to better replicate the clinical conditions: the specimens were manually mobilized by a physical therapist, a continuous load was applied, and C2 was rigidly fixed to represent the grip of a C2 stabilization during clinical assessments (side-bending stress test and rotation stress test). These are the first publications with head-C2 specimens which study the alar ligament influence on the kinematics. Applying the load continuously is also a novel aspect; stepwise loading has been commonly used in the studies focused on the role of the alar ligaments. But stepwise protocols lead to higher ROM, which has to be considered when comparing results among studies.

The in vitro motion was tracked by a **4-camera optoelectronic system**, which captured the position of reflective markers. To assess the motion with respect to the local coordinate system of the bones, a 3D measuring device (FaroArm) provided the coordinates of the reflective markers and the anatomical points of interest, and, with these landmarks, transformation matrices were applied between the reflective markers and the anatomical local coordinates. Furthermore, the applied load was tracked during the full ROM by a **6-axis load cell**: the specimens were vertically oriented on this load cell, with C2 attached. At the other end, a metallic handlebar was fixed to the skull to mobilize the specimens. Prior to each mobilization, a **neutral reference position** for the head was ensured: the Frankfurt plane (infraorbital foramen and external auditory meatus) was horizontal, as measured by a horizontal red-light laser in the lateral side of the specimen, and a vertical laser in front of a line in the center of the face.

The mobilizations in the three anatomical planes with the intact specimens have provided further values related to the upper cervical spine ROM to the literature.

But the novel aspects of these tests were the motions of these same specimens with other two conditions: unilateral alar ligament transection and C0-C1 stabilization.

The differences measured before and after **cutting one side of the alar ligaments** are of great interest from a clinical point of view. Practitioners need to assess the upper cervical spine stability prior to mobilizing these segments due to the risk of devastating neurovascular consequences. The side-bending stress test and the rotation stress test are commonly used, but some disagreements can be found in the literature. Related to the **side-bending stress test** (Chapter 3), these in vitro tests have shown lateral bending prior to the alar ligament transection, a motion previously associated with instability in the upper cervical spine. This means that lateral bending mobility may appear in healthy subjects. Additionally, in the **rotation stress test** (Chapter 4), one of the concerns is the maximum ROM to be expected by the practitioners. The in vitro tests of this thesis support the studies that provided values above 20° of unilateral axial rotation between the head and C2 in healthy populations; revealing that 20° might not be an appropriate cut-off value during the rotation stress test, as has been previously considered.

Related as well to these two clinical tests is the following: after the unilateral alar ligament transection, the ROM increased in both directions of the lateral bending and axial rotation. The ROM increases in lateral bending (right side: 33.5%; left side: 27.5%) and in axial rotation (right side: 13.7%; left side: 12.9%) corroborate the **bilateral effect that a unilateral alar ligament injury might show**, something that not all previous in vitro studies have supported. The new doubt raised after the quantification of these increases questions the ability of a practitioner to detect these changes below 2° in lateral bending or below 5° in axial rotation.

Considering only the ROM, these two tests would not have been sensitive in all the head-C2 specimens. Therefore, the **resistance perceived** is also important when diagnosing instability in the upper cervical spine; a reduced resistance was quantified in different points through the full ROM after the unilateral alar ligament transection. But caution must be taken when considering the resistance, as the increase in the resistance was only statistically significant in some cases (lateral bending: 0.75 Nm, both sides; axial rotation: 0.15 Nm and 0.30 Nm, only to the right side). Inter-subject variability due to anatomical variations should always be considered.

One step further considered was when the C0-C2 ROM was divided into C0-C1 and C1-C2 to analyze the **intersegmental behavior** (Chapter 5 and Chapter 6). By doing this, a different response was observed at the C1-C2 level after the right alar ligament transection: a slight increase was observed, from $1.6 \pm 1.2°$ to $1.8 \pm 1.1°$, while in the left side the increase was from $2.1 \pm 1.5°$ to $3.3 \pm 2.3°$. The same trend was detected in axial rotation, where the C1-C2 segment in the right rotation slightly changed from $32.3 \pm 9.3°$ to $32.4 \pm 9.4°$. Therefore, in clinical assessment, a more marked change could be expected in the C0-C1 mobility, although it must be considered that this change would most likely be below 2°.

The motion in the **sagittal plane** was also quantified, although no clinical test is applied with only a pure motion in flexion-extension. The most striking observation was after the alar ligament transection: although C0-C2 ROM increased, both in flexion (15.5%) and in extension (15.6%), the averages of the C0-C1 flexion ROM and the C1-C2 extension ROM decreased. This **intersegmental behavior** proves the importance of considering the individual segments to better understand the upper cervical spine mobility.

This intersegmental mobility and the relationship between C0-C1 ROM and C1-C2 ROM is interesting for clinical assessment: mobilizations in C0-C1 have increased C1-C2 mobility in patients, but a biomechanical background of this clinical observation was missing in the literature. The mobilizations in the three anatomical planes were repeated with an intact specimen after **stabilizing the C0-C1 segment** with two screws. And although the mobility was not fully restricted (being restricted by 74.4% in the sagittal plane, 76.9% in the frontal plane, and 90.9% in the transverse plane), an effect on the C1-C2 mobility was detected in axial rotation and in flexion, while the effect was not clear in lateral bending and extension. In a patient, the C0-C1 kinematics would be closely related to the tightening of the alar ligaments. Therefore, these in vitro observations would support the mobilization of C0-C1 for an indirect treatment of C1-C2 hypomobility, especially in axial rotation.

Studying the biomechanics of the spine under in vitro conditions can simulate the response of the spine during in vivo manual mobilization common in clinical practice. Specimens can be mobilized by a practitioner to track the full ROM considering the end-feel typically found in clinical mobilizations. The analyses presented in this thesis have widened the knowledge in the literature by being the **first in vitro simulation considering a unilateral alar ligament transection and the effects observed in the clinical tests that could clinically detect that injury (side-bending stress test and rotation stress test)**, with a C2 stabilization, as performed in the clinics during these two manual screening tests. Furthermore, this thesis has also provided the **first biomechanical study that analyzes the role of a C0-C1 restriction of movement on the C0-C2 kinematics**.

7.8.2 Conclusiones (in Spanish)

Los ensayos *in vitro* presentados en esta tesis, con muestras anatómicas cráneo-C2, han aportado a la literatura medidas del rango de movimiento (RDM) completo de manera continua en los tres planos anatómicos (inclinación lateral, rotación y flexión-extensión) incluyendo la sensación terminal del RDM de un fisioterapeuta manual. Además de analizar el movimiento segmentario, se estabilizó el segmento C0-C1 con tornillos para cuantificar su efecto en el movimiento del segmento inmediatamente inferior (C1-C2). Tras quitar dicha estabilización, los movimientos se repitieron en los tres planos anatómicos con una transección unilateral del ligamento alar derecho, para así analizar la influencia de los ligamentos alares en la cinemática de la columna cervical superior. Ampliar el conocimiento sobre todos estos aspectos relacionados con la biomecánica de la columna cervical superior es de gran valor desde el punto de vista clínico. Los profesionales que utilizan técnicas de terapia manual para evaluar la estabilidad de esta región cervical necesitan más evidencias científicas relacionadas con la respuesta del movimiento segmentario durante las técnicas manuales y la manera en la que una lesión en los ligamentos alares o la hipomovilidad en C0-C1 pueden alterar la respuesta del movimiento entre los segmentos de la columna cervical superior. Estos temas fueron abordados en los cuatro artículos de esta tesis.

Los resultados de estudios in vivo son más atractivos para los profesionales del ámbito clínico, ya que comparar de manera directa entre resultados *in vivo* e *in vitro* es difícil. Algunos aspectos pueden reducir la **biofidelidad** de los ensayos *in vitro*, como por ejemplo la eliminación de algunos tejidos blandos y la falta de reacción del cuerpo humano. Sin embargo, el uso de cadáveres para analizar la biomecánica humana es ampliamente aceptado y permite replicar lesiones, en este caso, de los ligamentos alares cortando un lado de esta estructura bilateral, y comparar, en la misma muestra, las alteraciones que este corte unilateral produce en la movilidad. Además, otras consideraciones se incluyeron en este protocolo *in vitro* para reproducir las condiciones clínicas: las muestras fueron movilizadas manualmente por un fisioterapeuta, realizando el movimiento de manera continua y fijando C2, quedando completamente inmóvil para representar la estabilización de C2 de las evaluaciones clínicas (test de estabilidad). Estas han sido las cuatro primeras publicaciones que no incluyen los niveles inferiores a C2 y que estudian la influencia del ligamento alar considerando únicamente la cinemática de la columna cervical superior. La aplicación continua del movimiento también ha sido un aspecto novedoso ya que estudios previos realizaban el movimiento de manera escalonada. Los movimientos escalonados en muestras anatómicas pueden alcanzar mayores RDM debido al comportamiento viscoelástico de los tejidos, un aspecto que debe considerarse a la hora de comparar los resultados de distintos estudios.

El movimiento *in vitro* fue capturado por un **sistema optoelectrónico** formado por cuatro cámaras, las cuales capturaron la posición tridimensional de marcadores reflectantes. Para evaluar el movimiento considerando sistemas de coordenadas locales en cada segmento óseo, se midieron con un sistema de medida 3D (FaroArm) las coordenadas de los marcadores reflectantes, así como coordenadas de referencias anatómicas en cada segmento; y con estas medidas, se calcularon matrices de transformación entre los marcadores y los sistemas de coordenadas locales de cada segmento. Además, la carga aplicada se midió de manera continua durante todo el RDM con una **célula de carga**. Los segmentos se orientaron de manera vertical sobre esta célula de carga, fijando la vértebra C2. En el otro extremo, un manillar metálico fue fijado al cráneo para movilizar las muestras. Antes de cada movilización se comprobó la **posición neutra** de la cabeza: el plano de Frankfurt (línea que une la parte inferior de la órbita y la parte superior del meato auditivo externo) se orientó horizontalmente con la línea horizontal de un láser proyectada sobre el lateral del cráneo, y otro láser vertical se colocó frente a una línea vertical en el centro de la cara.

Las movilizaciones en los tres planos anatómicos con los ligamentos alares intactos han aportado a la literatura más valores relacionados con el RDM de la columna cervical superior. Pero la parte innovadora de estos ensayos experimentales fue el cuantificar en las mismas muestras el movimiento bajo otras dos condiciones: con una transección unilateral en los ligamentos alares y estabilizando el segmento C0-C1.

Las diferencias detectadas entre antes y después de **cortar unilateralmente el ligamento alar derecho** son de gran interés desde el punto de vista clínico. Los profesionales clínicos necesitan evaluar la estabilidad de la columna cervical superior antes de movilizar estos segmentos debido al riesgo de daños neurovasculares devastadores. Tanto el test de estabilidad cervical superior de inclinación lateral como el de rotación se usan de manera común en la práctica clínica, pero en la literatura se pueden encontrar discrepancias en cuanto al uso de estas técnicas. En relación al **test de estabilidad de inclinación cervical superior** (Capítulo 3), estos ensayos *in vitro* han mostrado inclinación lateral antes del corte del ligamento alar, un movimiento que en estudios anteriores había sido asociado a inestabilidad en la columna cervical superior. Esto significa que existe la posibilidad de observar inclinación lateral en sujetos sanos. Además, respecto al **test de estabilidad de rotación cervical superior** (Capítulo 4), una de las preocupaciones entre los profesionales clínicos es el máximo RDM a esperar durante estas movilizaciones. Los ensayos *in vitro* de esta tesis doctoral apoyan a los estudios previos que indicaron valores superiores a 20° en la rotación unilateral entre la cabeza y C2 en sujetos sanos, revelando que el valor de corte de 20° usado en otros estudios anteriores puede no ser un límite apropiado para el test de rotación.

En relación a estos dos test clínicos: tras la transección unilateral del ligamento alar derecho, el RDM aumentó en los dos sentidos del movimiento en la inclinación lateral y en la rotación. El RDM aumentó en la inclinación lateral (lado derecho: 33.5%; lado izquierdo: 27.5%) y en la rotación (lado derecho: 13.7%; lado izquierdo: 12.9%), corroborando el **efecto bilateral que una lesión unilateral en los ligamentos alares puede mostrar**, algo sobre lo que no todos los estudios previos han estado de acuerdo. La duda que surge ahora tras cuantificar estos incrementos en el RDM es la posibilidad de poder detectar cambios inferiores a 2° en el test de inclinación lateral o inferiores a 5° en el test de rotación.

Considerando solo el RDM, estos dos test no habrían sido sensibles en todas las muestras ensayadas. Por tanto, la **resistencia percibida** por el profesional clínico es también importante a la hora de diagnosticar inestabilidades en la columna cervical superior; se cuantificó una reducción en la resistencia en diferentes instantes durante el RDM tras la transección del ligamento alar. Pero se debe tener precaución con esta observación, ya que este incremento en la resistencia fue solo estadísticamente significativo en algunos casos (inclinación lateral: 0.75 Nm, ambos sentidos; rotación: 0.15 Nm y 0.30 Nm, solo hacia el lado derecho). La variabilidad entre sujetos debido a las variaciones anatómicas debe ser siempre considerada.

Un paso más allá se tomó al considerar los movimientos entre cada segmento: C0-C1 y C1-C2, para analizar el **comportamiento segmentario** (Capítulo 5 y Capítulo 6). Haciendo esto, se observó una respuesta diferente en C1-C2 tras la transección del ligamento alar derecho: hubo un pequeño aumento en el RDM, de $1.6 \pm 1.2°$ a $1.8 \pm 1.1°$, mientras que en el lado izquierdo el incremento fue de $2.1 \pm 1.5°$ a $3.3 \pm 2.3°$. Esta misma tendencia se observó en la rotación axial, donde C1-C2 hacia la derecha mostró un incremento mínimo, de $32.3 \pm 9.3°$ a $32.4 \pm 9.4°$. Por lo tanto, en diagnósticos clínicos, un cambio mayor podría ser esperado en la movilidad de C0-C1, aunque debe considerarse que este cambio sería probablemente inferior a 2°.

El movimiento en el **plano sagital** también se cuantificó, aunque ningún test clínico se aplica únicamente en flexión-extensión. La observación más llamativa fue después del corte en el ligamento alar, ya que aunque el movimiento de C0-C2 aumentó, tanto en flexión (15.5%) como en extensión (15.6%), los valores medios de la flexión de C0-C1 y de la extensión de C1-C2 disminuyeron. Este **comportamiento segmentario** muestra la importancia de considerar los segmentos de manera individual para un mejor entendimiento de la movilidad de la columna cervical superior.

La movilidad segmentaria y la relación entre el RDM de C0-C1 y de C1-C2 es interesante de cara a los diagnósticos clínicos: movilizaciones en C0-C1 han incrementado la movilidad en C1-C2 en pacientes, pero en relación a esto falta en la literatura una base científica que explique el mecanismo biomecánico de esta relación en la columna cervical superior. Las movilizaciones en los tres planos anatómicos se repitieron con los ligamentos alares intactos y **estabilizando el segmento C0-C1** con dos tornillos. Aunque la movilidad no fue restringida por completo (se restringió un 74.4% en el plano sagital, un 76.9% en el plano frontal y un 90.9% en el plano transverso), se detectó un efecto en la movilidad de C1-C2 en la rotación y en la flexión, sin estar claro el efecto en la inclinación lateral y en la extensión. En los pacientes, la cinemática de C0-C1 estaría estrechamente relacionada con el grado de tensión de los ligamentos alares. Por tanto, las observaciones de estos ensayos *in vitro* apoyan las movilizaciones del segmento C0-C1 en tratamientos indirectos cuya finalidad es mejorar la movilidad de C1-C2; especialmente, en la rotación axial.

El estudio de la biomecánica de la columna vertebral en condiciones *in vitro* puede simular la respuesta de la columna en movilizaciones manuales comunes en la práctica clínica. Los segmentos pueden ser movilizados por un profesional clínico para cuantificar el RDM completo considerando la sensación terminal encontrada típicamente en las movilizaciones clínicas. Los análisis presentados en esta tesis han ampliado el conocimiento existente al ser **las primeras simulaciones *in vitro* considerando una transección unilateral del ligamento alar y estudiando posteriormente los efectos observados en las movilizaciones que se usan clínicamente para detectar inestabilidad cervical superior (test de estabilidad de inclinación cervical superior y test de estabilidad de rotación cervical superior)**, incluyendo la estabilización de C2 al igual que en las pruebas clínicas. Además, esta tesis doctoral también ha aportado a la literatura **el primer estudio biomecánico que analiza el papel de una restricción del movimiento en C0-C1 en la cinemática de C0-C2**.

Appendix A

Figures' Sources and Copyrights

This appendix provides a list of the sources from where the images presented in this thesis were obtained:

1. Figure 1.1: This is a region from the original image which showed the full cervical spine and skull, downloaded from Wikimedia Commons. Licenser: "BodyParts3D, The Database Center for Life Science licensed under CC Attribution-Share Alike 2.1 Japan".
   https://commons.wikimedia.org/wiki/File:C3_lateral.png,
2. Figure 1.2: Downloaded from Wikimedia Commons.
   https://commons.wikimedia.org/wiki/File:Vertebra_-_atlas.jpg
3. Figure 1.3: Downloaded from Wikimedia Commons.
   https://commons.wikimedia.org/wiki/File:Vertebra_-_atlas,_axis_(superior).jpg
4. Figure 1.4: This is a region from the original image which showed the full cervical spine, downloaded from Wikimedia Commons (provided by Radiopaedia).
   https://commons.wikimedia.org/wiki/File:Vertebral_artery_3D_Lateral.jpg
5. Figure 1.5: This image is in the public domain as it was published in 1918. It appeared in "Anatomy of the Human Body", by Henry Gray.

Appendix B

Contribución del doctorando en los artículos en coautoría

La tesis incluye las siguientes cuatro publicaciones. Todas ellas están relacionadas con la movilidad de la zona cervical superior en inclinación lateral (n.º 1), rotación (n.º 2), los tres planos anatómicos (a nivel segmentario, n.º 3), y, por último, el efecto del movimiento entre el hueso occipital y atlas en la cinemática de la zona cervical superior (n.º 4). En los tres primeros artículos se analiza el movimiento antes y después de seccionar el ligamento alar, analizando cómo este ligamento afecta a la cinemática. El cuarto artículo se centra en el movimiento cervical con y sin fijación del segmento occipital-atlas, lo que está relacionado con la puesta en tensión del ligamento alar.

1. Hidalgo-García, C., Lorente, A.I., Rodríguez-Sanz, J., Tricás-Moreno, J.M., Simon, M., Maza-Frechín, M., Lopez-de-Celis, C., Krauss, J. and Pérez-Bellmunt, A., 2020. Effect of Alar Ligament Transection in Side-bending Stress Test: A Cadaveric Study. *Musculoskeletal Science and Practice*, 46, p.102110. https://doi.org/10.1016/j.msksp.2020.102110
   Impact Factor: 2.520, Rehabilitation (Q2)

2. Hidalgo-García, C., Lorente, A. I., Lucha-López, O., Auría-Apilluelo, J. M., Malo-Urriés, M., Rodríguez-Sanz, J., López-de-Celis, C., Maza-Frechín, M., Krauss, J. and Pérez-Bellmunt, A., 2020. The Effect of Alar Ligament Transection on the Rotation Stress Test: A Cadaveric Study. *Clinical Biomechanics*, 80, p.105185.
   https://doi.org/10.1016/j.clinbiomech.2020.105185
   Impact Factor: 2.063, Biomedical Engineering (Q3)

3. Lorente, A.I., Hidalgo-García, C., Rodríguez-Sanz, J., Maza-Frechín, M., Lopez-de-Celis, C. and Pérez-Bellmunt, A., 2021. Intersegmental Kinematics of the Upper Cervical Spine: Normal Range of Motion and its Alteration After Alar Ligament Transection. *Spine*, 46 (24), p.E1320.
   https://doi.org/10.1097/BRS.0000000000004167
   Impact Factor (2020): 3.468, Orthopedics (Q1)

4. Hidalgo-García, C., Lorente, A.I., López-de-Celis, C., Lucha-López, O., Malo-Urriés, M., Rodríguez-Sanz, J., Maza-Frechín, M., Tricás-Moreno, J.M., Krauss, J. and Pérez-Bellmunt, A., 2021. Effects of Occipital-atlas Stabilization in the Upper Cervical Spine Kinematics: an in Vitro Study. *Scientific Reports*, 11(1), p.1-13. https://doi.org/10.1038/s41598-021-90052-6
   Impact Factor (2020): 4.379, Multidisciplinary Sciences (Q1)

Contribución del doctorando

(A) Diseño del protocolo experimental para la realización de los ensayos con material cadavérico: medición del movimiento (sistema de captura Vicon), obtención de coordenadas en las vértebras y en el cráneo para tener referencias anatómicas (brazo de medición FaroArm) y medición de la carga aplicada (célula de carga).

(B) Redacción de la hoja de control (checklist) para los ensayos experimentales, con una lista de todos los pasos a seguir.

(C) Preparación de la sala y acondicionamiento del área de trabajo de cara a los ensayos experimentales (realizados en el Laboratorio del Impacto – I3A): instalación de las cámaras del sistema de captura de movimiento Vicon y calibración del sistema, preparación de la célula de carga, colocación de dos láseres (para el posicionamiento de las muestras) e instalación de la célula de carga y del brazo de medición FaroArm. Revisión de los protocolos de seguridad para el manejo de muestras biológicas.

(D) Obtención de los datos durante los ensayos: las capturas de movimiento con Vicon, las medidas de la célula de carga y las coordenadas de FaroArm.

(E) Reconstrucción de las trayectorias de los marcadores del sistema Vicon (con el software propio del sistema, Nexus).

(F) Preparación de códigos en Matlab para el procesamiento de todos los datos (captura de movimiento, célula de carga y brazo de medición FaroArm).

(G) Cuantificación del movimiento occipital-axis y axis-atlas, y su relación con la carga aplicada durante todo el rango de movimiento. Obtención de tablas y gráficas para la presentación de los resultados en los cuatro artículos.

(H) Redacción completa del artículo n.º 3 y envío a la revista *Spine* como primer autor. Redacción junto al Dr. César Hidalgo García de los otros tres artículos.

(I) Participación en el proceso de revisión de los cuatro artículos tras los comentarios de los revisores de las revistas: modificación de los artículos y redacción de las respuestas para los revisores.

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