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Investigation of meningomyovertebral structures within the upper cervical epidural space: a sheet plastination study with clinical implications

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Abstract

BACKGROUND CONTEXT: Over the past two decades, soft-tissue structures communicating with the dura mater within the epidural space have become the focus of many anatomical and histopathologic studies. The relationship between these bridging structures has yet to be evaluated *in situ*.

PURPOSE: This is the first study that used E12 sheet plastination to investigate the epidural space of the upper cervical spine *in situ* and its associated bridging structures. Given the complexity of this space, this study may prove useful to clinical anatomists and surgeons who operate within this region.

STUDY DESIGN: Anatomical and microscopic analyses of structures that communicate with the dura mater within the upper cervical region were carried out.

METHODS: Gross dissection in conjunction with microscopy was used to evaluate bridging communications of the upper cervical spine in 10 cadavers. To evaluate the *in situ* arrangement of these structures, E12 sheet plastination was used on 13 cadavers.

RESULTS: In all 23 specimens, suboccipital fascia coalesced with the dorsal meningovertebral ligament of the atlas, and inserted directly into the posterior surface of the dura as a single but separable laminar layer. At the level of the atlantoaxial interspace, suboccipital fasciae combined and coalesced with the dorsal meningovertebral ligament of the atlas and the axis. These structures inserted into the posterior surface of the dura mater as a single but separable layer. Microscopy validated these findings and E12 sheet plastination revealed the *in situ* organization of these soft-tissue structures. E12 sheet plastination also provided new information on dural arrangement at the craniocervical junction, which was observed to be composed of periosteum from the occiput but consisted mainly of deep fascia from the rectus capitis posterior minor.

CONCLUSIONS: E12 sheet plastination has provided *in situ* visualization of bridging structures within the cervical epidural space and offers new insight into these structures, as well as the composition and arrangement of the posterior atlantooccipital membrane and cerebrospinal dura at the craniocervical junction. This study aims to expand on the anatomical understanding of the upper cervical region while defining structures that may reduce neurosurgical complications, and aid in the understanding of the pathophysiology of certain neurogenic disorders.

Keywords: Cervical spine; Dural lacerations; Epidural space; Meningovertebral ligaments; Suboccipital muscles; Myodural bridge; PAO membrane

FDA device/drug status: Not applicable.

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The authors declare no conflicts of interest associated with this study.

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Introduction

The epidural space is occupied by many soft-tissue connections that serve to bridge the dura with surrounding ligamentous, muscular, and osseous structures [1–9]. With respect to epidural connective tissue bands, most studies focus on those of the lumbosacral region [6,10–14]. The well-documented anterior dural (Hofmann) ligaments extend from the dura mater to the posterior longitudinal ligament and were first thought to exist exclusively within the anterior epidural space of the lumbar region [13]. Later studies [14] revealed that these structures also exist from the seventh cervical to the fifth lumbar vertebrae. Existence of anterior dural ligaments (Trolard) within the sacral epidural space has been reported as well [10]. Whereas the majority of anterior dural ligaments insert into the posterior longitudinal ligament, others traverse deeper to coalesce with the endosteum of the surrounding vertebral structures [12].

Although the meningovertebral ligaments of the lumbar spine have been reported extensively, the origin of upper cervical posterior epidural ligaments remains unclear [3,15–17]. In 1995 Shinomiya et al. [17] reported posterior epidural ligaments linking the dura mater to the ligamentum flavum at levels of the first cervical vertebra (C1) to the seventh cervical vertebra. Their work emphasizes an abundance of ligaments at levels C1 to the second cervical vertebra (C2) and sparsity at the level of the sixth and seventh cervical vertebrae. Connections at the third (C3) through seventh cervical vertebrae were confirmed later in 1996 [18]. Contrary to Shinomiya et al.'s findings, an earlier study [3] examined the posterior intervertebral spaces and briefly described epidural ligaments at the level of C1, which communicate the periosteum of the atlas to the dura mater. In 2014, cervical meningovertebral ligaments throughout the cervical epidural space were again isolated, mainly extending from the ligamentum flavum to the posterior dural surface [9].

This study examines the relationship between the dorsal meningovertebral ligaments of the first two cervical vertebrae and their adjacent myodural structures. To confirm a true anatomical connection, the cervical epidural space was examined grossly and validated using microscopy. These epidural structures were also observed *in situ* using E12 sheet plastination. Should the dorsal meningovertebral ligaments interact with myodural attachments, they may be subject to the same pathologic complications hypothesized in the literature [1,7,8,16,17]. Considering dural laceration is one of the most common complications of spinal surgery [9], knowledge of surgical anatomy of the dorsal meningovertebral ligaments and their variations could provide clues to improve the risk of surgical and postsurgical complications [19,20].

Materials and methods

A total of 23 embalmed adult cadaveric specimens (16 men and 7 women, aged 54–89 years) were examined in this study. Ten specimens (6 men and 4 women, aged 54–86 years) were selected at random from the Department of Anatomical Sciences at Logan University, and underwent gross dissection followed by examination with light microscopy. Thirteen specimens (10 men and 3 women, aged 67–89 years), prepared for E12 sheet plastination procedure, were obtained from the Department of Anatomy and Structural Biology at Otago University, School of Medical Sciences. Specimens with signs of anatomical variation, prior surgery, or trauma in the area of interest were excluded from this study. All guidelines for use of cadaveric material in research were followed.

Gross dissection

Initially, the trapezius and splenius capitis muscles were reflected posteriorly from the superior nuchal line to expose deep suboccipital muscles. To verify the transverse atlantoaxial and atlantooccipital myodural connections, the posterior elements of the first two cervical vertebrae, the rectus capitis posterior minor (RCPmi), rectus capitis posterior major (RCPma), and obliquus capitis inferior (OCI) were all identified and preserved.

In all 10 specimens, a partial laminectomy from C3 through the sixth cervical vertebrae was performed using a Stryker Autopsy 810 saw (Stryker, Kalamazoo, MI, USA). With a scalpel, the RCPma, OCI, and RCPmi were removed from their bony attachment sites bilaterally. Firm posterior traction was applied on these muscular structures to verify whether they maintained their myodural attachments.

Once the axis was completely denuded of these muscular structures, the flat end of the scalpel handle was inserted into the horizontal plane of the inferior aspect of the atlantoaxial interspace to protect the epidural structures. A Dremel 200-1/15 two-way rotary (Robert Bosch Tool Corporation, Mt. Prospect, IL, USA) with a 426 1-1/4 inch fiberglass-reinforced cutoff wheel attachment was then used to perform a midsagittal cut through the bifid process of the C2 vertebra. A second cut was performed on the far lateral side of the lamina of the C2 vertebra. Contralaterally, the same far lateral approach was performed to separate C2. The same process was applied to the posterior arch of the atlas. Firm posterior traction was applied on the sections of atlas and axis to verify if the osseous structures remained attached to the dura mater. Examination of the dorsal surface of the posterior atlantooccipital (PAO) membrane was carried out to establish the architecture of the fibrous tissue composition. The ventral surface of the PAO membrane was also examined for possible attachments to the posterior aspect of the dura. Gross anatomical data were recorded using a Nikon D-40 camera with an attached Nikon DX AF-S Nikkor 18–55 mm 1:35-5.6 GII lens (Nikon Corporation, Chiyoda-ku, Tokyo, Japan).

Microscopy

Where bridging connective tissue communicated with the dural sleeve, a 2 cm × 2 cm section of the dura mater was

excised. Muscles, bone, and dura were placed as one continuous piece into a neutral buffered solution. Samples were labeled and sent to the Department of Pathology at St. Louis University for histologic examination. Each 2 cm × 2 cm tissue section was dehydrated and infiltrated with paraffin in a Tissue Tek VIP auto-processor (Sakura Fintek USA Inc., Torrance, CA, USA), embedded in paraffin within sectioning cassettes, trimmed and sectioned at 5 μm. Sections were collected on glass slides, dried at 36°C overnight, stained with hematoxylin and eosin (H&E) in a Leica Autostainer XL (Leica Biosystems Inc., Buffalo Grove, IL, USA), and coverslipped in Permount (Fisher Scientific, Pittsburgh, PA, USA) under #1.5 glass coverslips. Stained sections were evaluated and photographed using an Olympus BX 41 light software (Olympus America, Center Valley, PA, USA). Images were taken with a 4× or 10× objective and stitched with Image Composite Editor software (Microsoft Research, Redmond, WA, USA).

E12 sheet plastination

Specimens examined were prepared as sets of 2-mm-thick epoxy resin slices using the E12 sheet plastination technique [21]. Preparation required embalming of cadaveric specimens followed by freezing at −85°C for 48 hours. Once frozen, each specimen was sectioned, then dehydrated and degreased by immersing tissue slices into acetone chilled at −25°C. Acetone concentration was gradually increased over a period of 20–22 weeks until the dehydration and degreasing process was approved based on tissue clarity, shrinkage, and preservation. Next, vacuum impregnation of resin mixture was conducted under cryogenic conditions (−8 to 0°C) over a 2-day period. Impregnation was complete when bubbles failed to appear from tissue specimens. Sections were then laminated by being placed in a warm water-bath and then in an oven set at 35°C for 24 hours to achieve solidification.

Translucent plastinations were initially examined on a radiographic light box. Each slice was subsequently reviewed macroscopically under low magnification (range 0.63×–1.25×) with a Leica MZ8 Stereoscopic Dissecting Microscope (Leica Microsystems Inc., Buffalo Grove, IL, USA). The fibrous architecture and distribution of structures within the intervertebral and epidural spaces between the levels of the craniocervical junction to the level of C3 were investigated and recorded. Photographs were captured with a Nikon Coolpix 990 Digital Camera (Nikon Corporation, Chiyoda-ku, Tokyo, Japan).

Results

The same fibrous connective tissue configurations were found in all samples examined, with only minor individual variations.

Gross dissection

The deep and lateral fascia of the RCPmi appeared to be continuous with the PAO membrane. The superior aspect of the PAO membrane extended from the posterior border of the foramen magnum. Anteriorly, this periosteal tissue communicated and seemed to merge with the dura mater at the level just below the atlas. The deep fascia of the RCPmi appeared to blend with the PAO membrane but traversed anteroinferiorly to form the atlantooccipital myodural bridge. This bridging structure entered into the epidural space where it coalesced with an extension of periosteum originating from a ligamentous structure expanding from the surface of the posterior arch of the atlas. This extension was identified as the dorsal meningovertebral ligament of C1. The myodural bridge and dorsal meningovertebral ligament fused along their distal length and had a common insertion site on the posterior surface of the dura mater. The two layers were easily separable but maintained a common dural insertion point. The directional position of this structure was oblique and anteroinferior. All structures involved in this soft-tissue communication and their attachment sites were resistant to manual traction.

At the level of the atlantoaxial interspace, the epimysium of the RCPma and OCI attached in parts to the lamina of the axis but mainly combined to form fibrous bands that traversed the atlantoaxial interspace. These fibrous cord-like bundles haphazardly passed between two thin strips of the ligamentum flavum. The ligamentum flavum was significantly reduced in their lateral dimensions between C1 and C2 in comparison with other vertebral levels (Fig. 1A and B). Once the fascial bands merged within the epidural space, it was easily identified as the atlantoaxial myodural bridge. Notably, the posterior branch of the second spinal nerve pierced through this tissue. The atlantoaxial myodural bridge coalesced with a ligament-like structure, which expanded from the lamina of the axis. This structure was identified as the dorsal meningovertebral ligament of C2. Less obvious was an analogous structure extending from the inferior pole of the posterior arch of C1 and ligamentum flavum of C1/C2 (Fig. 1C). These layers haphazardly expanded out and attached to the fibrous bundles of the myodural bridge as it passed between C1 and C2. All layers of the soft-tissue structure that inserted into the dura were easily separable but maintained a common dural insertion point. The directional position of these structures within the atlantoaxial epidural space was oblique and anteroinferior. All structures involved in this soft-tissue communication and their attachment sites were resistant to manual traction (Fig. 2).

Microscopy

Microscopy of the atlantooccipital interspace with H&E stain confirmed dense tissue extending into the epidural space as a continuation of fascia from the RCPmi. This soft-tissue structure merged with a dense connective tissue tract extending from the posterior arch of C1. The two structures formed a single laminar layer and inserted into the posterior surface of the dura mater.

Histologic analysis of the atlantoaxial interspace with H&E stain confirmed a band of tissue extending from the anterior

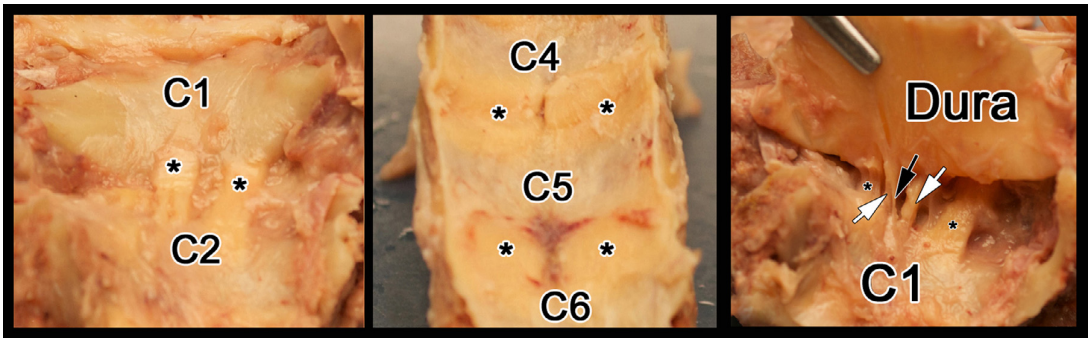

Fig. 1. (Left, Middle) Standard laminectomy was conducted to compare the ligamentum flavum between the atlas (C1) and the axis (C2) with cervical vertebrae at lower levels (C4, C5, and C6). Bridging structures were excised to demonstrate the area of interest. The ligamentum flavum (asterisks), at the level of the atlantoaxial interspace (Left), reveals a significant decrease in lateral dimensions when compared with the ligamentum flavum at the lower cervical levels (Middle). This significant decrease in width allows for patency between the atlas and axis to provide passage of the atlantoaxial myodural bridge to breach into the epidural space and communicate with the cervical dura mater. (Right) Photograph reveals gross dissection of a superior to inferior view of the atlantoaxial interspace from the perspective of the epidural region. The space between the atlas (C1) and the axis contains bundles of fibrous tissue passing between two thin strips of ligamentum flavum (asterisks). These bands consist of deep fascia from the RCPma and OCI (white arrows), and ligamentous components extending from the atlas (black arrow) and axis. These bands of tissue fuse and communicate with the spinal dura.

surface of C2 lamina. This bridging tissue traversed through the epidural space and merged with the epimysium of the OCI. The two structures coalesced to form a dense band, which ultimately inserted into the posterior surface of the dura mater (Fig. 3).

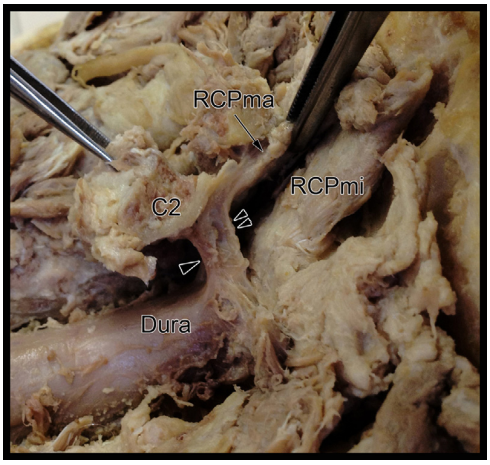

Fig. 2. Photograph reveals posterior view of the suboccipital region following hemi-laminectomy of the axis (C2) without excision of the suboccipital muscles. This method allows for proper identification of the elements that communicate with the cervical dura mater. Two separate bands of tissue extending from the RCPma and atlas are observed. One fibrous band, identified as the dorsal meningovertebral ligament of C2 (single arrowhead), extends from the lamina of the axis and attaches to the cervical dura. A second adjacent fibrous band of tissue, identified as the atlantoaxial myodural bridge (double arrowheads), extends from the RCPma, runs adjacent to the dorsal meningovertebral ligament of C2, and connects to the dura mater. Firm posterior traction with forceps of osseous and soft-tissue structures suggests a strong attachment to the dura mater. RCPma, rectus capitis posterior major; RCPmi, rectus capitis posterior minor.

E12 sheet plastination

All 13 specimens that underwent E12 sheet plastination revealed that the fascia from the RCPmi contributed to the vascular sheath of the vertebral artery laterally and to the majority of the PAO membrane. The superior aspect of the PAO membrane contained a small continuation of periosteum from the posterior aspect of the occiput. This layer of periosteum continued in an anteroinferior oblique angle, surrounded the internal vertebral plexus, and coalesced with periosteal layers extending from the anterior and inferior aspects of the occiput. The three periosteal layers created the most posterior layer of the cerebrospinal and spinal dura. These three periosteal layers coalesced and became indistinguishable from the spinal

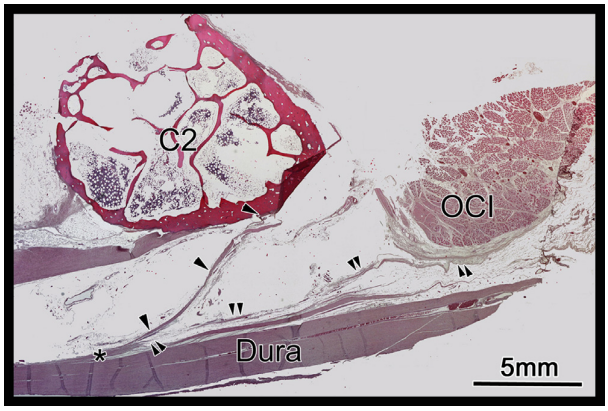

Fig. 3. Microscopy under low magnification of the epidural space at the level of the axis (C2) that underwent hematoxylin and eosin stain. The deep fascia of the OCI (double arrowheads) enters the epidural space to merge with a ligamentous layer (single arrowheads) extending from the axis. The two layers communicate (asterisk) and blend with the dura. Scale bar = 5 mm. OCI, obliquus capitis inferior.

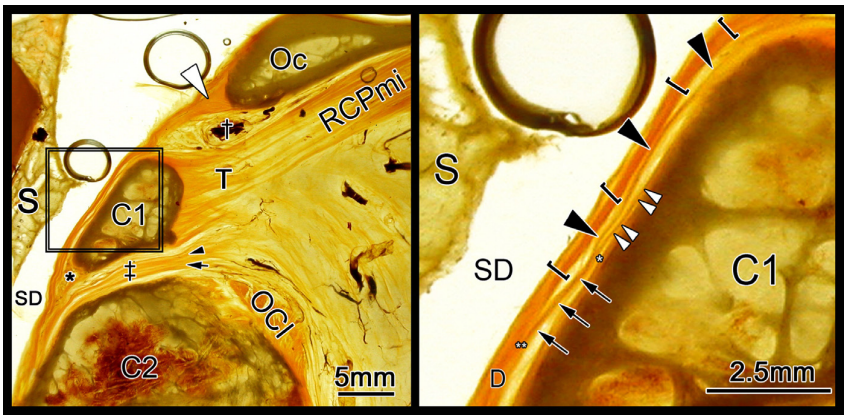

Fig. 4. (Left) Photograph of a sagittal epoxy resin slice revealing the atlantooccipital and atlantoaxial interspaces. Atlantooccipital space: The RCPmi sends tendinous fibers (T) mainly to the posterior arch of the atlas (C1). The area between the fibers of the RCPmi and multilayered periosteum of the occiput (white arrowhead) houses the vertebral artery (†). The anterior, middle, and posterior layers of occipital periosteum merges with the cerebrospinal dura mater. Atlantoaxial space: Tendinous fibers of the RCPma (black arrowhead) and OCI (black arrow) merge to form a fibrous membrane (‡) that occupies the vertebral interspace between the atlas and axis (C2). Periosteum from the inferior border at the posterior arch of the atlas (asterisk) coalesces with this membrane within the epidural space to form a band of tissue that communicates with the spinal dura mater. The highlighted area signified by the box represents the region of magnification in panel B. Scale bar = 5 mm. (Right) Magnified section of panel A. Deep fascia of the RCPmi (black arrowhead) traverses through the epidural space and merges (single asterisk) with the periosteum (double white arrowheads) of the atlas (C1). The two layers form a single membrane (black arrows) that attach (double asterisks) to the posterior aspect of the spinal dura (D). The multiple layers of the occipital periosteum merge with the spinal dura (brackets). The thicker laminae of occipital periosteum separate and reconnect to the thin layer of spinal dura along this region because of separation of laminar planes during plastination technique. They become an inseparable unit at the level of the third cervical vertebra. Scale bar = 2.5 mm. RCPmi, rectus capitis posterior minor; RCPma, rectus capitis posterior major; OCI, obliquus capitis inferior; SD, subdural space; S, spinal cord.

dura at the level of C3. All upper cervical bridging structures merged with this structure before inserting directly into the dura mater.

The RCPmi fascia passed into the epidural space at the superior border of the atlas, descended, and then merged with the dorsal meningovertebral ligament of C1. As what appeared to be a single layer, this combined structure passed through the epidural space in an anteroinferior direction and inserted into the posterior surface of the cervical dura mater (Fig. 4A and B).

In 12 of 13 specimens, deep fasciae from the RCPma and OCI passed into the atlantoaxial interspace. In certain sagittal slices, the ligamentum flavum was noted between these vertebrae. Within the epidural space, a complex network of fibrous layers extended from the inferior pole of C1 to merge with the atlantoaxial myodural bridge (Fig. 4A). This membrane, composed of periosteum and fasciae, continued inferiorly within the epidural space and coalesced with the periosteum of C2. Ultimately, all of these structures inserted into the posterior surface of the cervical dura mater. There was evidence of these laminae splitting and rejoining, but they inserted at the same point into the cervical dura. In one specimen, the area of interest at the atlantoaxial epidural space was blocked from examination because of shifting and overlapping of cadaveric tissue during the sheet plastination procedure.

Discussion

The epidural space of the upper cervical spine is a very complex region containing many connective tissue layers that merge throughout their respective courses. This investigation delineates the relationship between the recently reported myodural bridging structures and the dorsal meningovertebral ligaments that occupy C1 and C2 vertebral interspaces. In the present study, the dorsal meningovertebral ligaments originated as dense connective tissue from the atlas and axis, which traversed the epidural space, merged with fascial components of the atlantooccipital and atlantoaxial interspaces, respectively, and inserted into the posterior aspect of the cervical dura mater (Fig. 5A and B). E12 sheet plastination has provided *in situ* visualization of these structures and offers new insight into the composition and arrangement of the PAO membrane and cerebrospinal dura at the craniocervical junction.

This study confirms recent reports regarding fascial components of suboccipital muscles, which enter the epidural space and insert into the dura [1,3,7,9,16]. The RCPmi has been repeatedly shown to emit an anterior fibrous band that bridges across the posterior epidural space at the level of the atlantooccipital interspace [1,3,22,23]. The RCPma and the OCI emit similar fibrous bands that traverse the posterior epidural space at the level of the atlantoaxial interspace [7,8,16,22,23]. All three of these soft-tissue bands observed inserted into the cervical dura adjacent to the C1 and C2 dorsal meningovertebral ligaments in this study.

The dural connections identified in this study may serve to maintain position of the spinal cord in the vertebral canal during movements of the head and neck. Considering the meningovertebral structures attach directly to osseous components, they may provide greater stability than their fascial

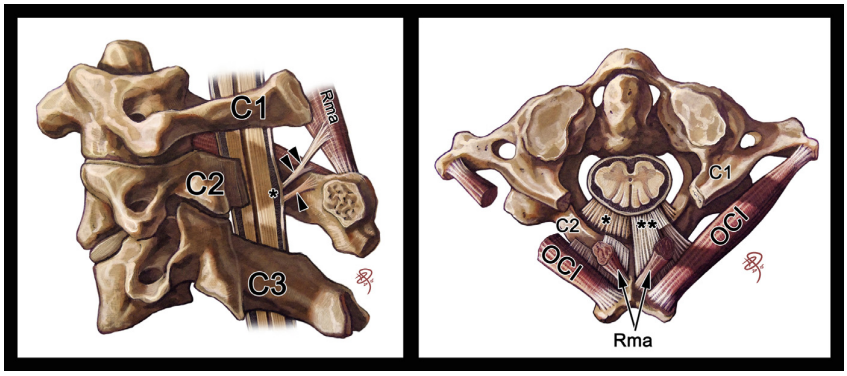

Fig. 5. (Left) Illustration depicts a lateral view of the first through third cervical vertebrae (C1–C3). The left lamina of C2 is excised to reveal the contents of the vertebral canal and epidural bridging contents. The atlantoaxial myodural bridge (double arrowheads), which consists of deep fascia from the RCPma and OCI, inserts into the dura mater (asterisk) at a level inferior to the atlantoaxial junction. The dorsal meningovertebral ligament of C2 (single arrowhead) extends from the anterior surface of the axis' lamina and inserts into the dura mater adjacent and inferior to its closely related myodural component. (Right) Illustration depicts a superior to inferior view of the upper cervical vertebral canal and the close relationship of the atlantoaxial epidural bridging contents. The OCI and RCPma extend their myodural bridge (double asterisks) into the vertebral canal. This fascial structure extends through the epidural space to communicate with the cervical dura mater. Partial excision of the posterior arch of the atlas (C1) and musculofascial components reveal the more inferior dorsal meningovertebral ligament of C2 (single asterisk). RCPma, rectus capitis posterior major; OCI, obliquus capitis inferior.

counterparts. These "bridging" structures may also serve to maintain patency of the subarachnoid space, allowing proper cerebrospinal fluid outflow from the cisterna magna [1,7,8,16]. Findings in this study support the aforementioned physiologic and stabilizing considerations provided that the greatest amount of head and neck flexion and rotation occurs at the craniocervical and atlantoaxial joints, respectively [19]. The biomechanical importance of the myodural structures becomes apparent during contraction of the deep suboccipital muscles. As the RCPmi and RCPma extend the craniocervical junction, tension of the fascial bridge pulls the spinal dura posteriorly, preventing dural enfolding. As the OCI and RCPma rotate the atlantoaxial junction, the fascial bridges associated with these muscles pull the spinal dura ipsilaterally, preventing torsional distortion of dura. The ligamentous structures identified in this study, which merge with these fascial bridges, may amplify the abovementioned effects by providing synergistic tension on the dura during extension and rotation. As the head and neck return to anatomical position, these ligamentous structures may provide support and proper elastic recoil to return the dura and associated fascial structures back to baseline. Tensile forces acting on dura within the epidural space may also indirectly influence dentate ligaments within the subarachnoid space. A system of this nature would allow for dura and its contents to behave in a synchronized manner along with vertebral movement. This hypothesis is supported by a recent analysis of the upper cervical dura, in which the quantity and orientation of elastin fibers from caudal to cranial were found to vary [24]. Functioning as anchoring points for epidural ligaments, these elastic properties may create a spectrum of varying tensile forces across the dura, which coincide with the extent of movement at each vertebral level [24,25].

Positive immunofluorescence of anti-neurofilament antibodies in the myodural bridge supports the hypothesis that neurons are located within the myodural structures [15,16]. In this study, the posterior branch of the second spinal nerve was seen to pierce the atlantoaxial myodural bridge, which may be the source of neural tissue identified in previous studies. Considering the posterior root of the second spinal nerve contains afferent signals to higher centers, branches of this nerve may modulate upper cervical dural tension and initiate sensory reflexes to suboccipital muscles [16]. Of clinical significance, contraction of the suboccipital muscles may cause the fascial bridging components of the OCI and RCPma to compress the second spinal nerve leading to an additional source of occipital neuralgias [26].

Aside from their proposed physiologic functions, these bridging structures may be involved in a wide variety of clinical manifestations. The literature suggests these bridging structures serve a vital role in anchoring the dura mater and protecting against spinal cord compression [7,8,16,17]. Analogous to the support offered by subarachnoid denticulate ligaments, these fibrous epidural bands may provide mechanical function in tethering the dura, and its contents, in place. Anchored by osseous structures, their disruption may be even more detrimental to spinal cord posture than ligaments described in the Shinomiya studies [17,18]. The lack of posterior epidural ligaments, or loss of their integrity, may destabilize the spinal cord by disrupting the cervical myo-reflexive response [24,25]. This mechanism is thought to result in anterior translation of the spinal cord during flexion movements of the head and neck [17]. Micro-strains and trauma of the RCPma, RCPmi, and OCI muscles as well as tendons may cause clonus contraction of the muscle and stimulate the pain-sensitive dura, generating cervicogenic headaches [27,28].

These bridging communications may also refer pain via the trigeminal sensory distribution and may also be involved in spontaneous cerebrospinal fluid accumulations within the atlantoaxial interspace [1,2,16,29–31]. The dense connective tissue component of the upper cervical dorsal meningovertebral ligaments firmly attaches to the dura mater in the same manner as the cervical myodural structures, and therefore may be subject to similar pathologic complications such as destabilization of the spinal cord, cervicogenic headaches, trigeminal nerve pain distribution, and spontaneous cerebrospinal fluid accumulations within the atlantoaxial interspace.

Using E12 sheet plastination technique in this study made it possible to examine the detailed anatomy of the PAO interspace *in situ*. It was believed that the PAO membrane consisted of periosteum from the occiput and the arch of the atlas [32]. The results of this study suggest that this concept may not be correct. Superiorly, the "PAO membrane" consisted of periosteum of the occiput, whereas inferiorly it formed part of the dura at the cerebrospinal junction rather than attaching to the posterior arch of the atlas. The results of this study reveal that the so-called PAO membrane was found to be mainly formed by the deep fascial layer of the RCPmi with lateral contributions derived from perivascular sheaths of the vertebral artery. These findings are consistent with a previous study which describes the anatomy of the atlantooccipital interspace [4]. The results of the present study reveal that the putative PAO membrane appears to be more complex and intimately involved with upper cervical bridging structures than previously described. As this multilayered soft-tissue component passes inferiorly through the epidural space, bridging soft-tissue components become layered onto the posterior aspect of the PAO membrane before their full integration into the spinal dura at the C3 vertebral level. This anatomical arrangement of the PAO membrane may indicate a superiorly located anchor point for all upper cervical bridging epidural structures. Acting as a common attachment site, the PAO membrane would allow for tensile forces across all upper cervical bridging structures to act in a summated synchronized manner during head and neck movement. This morphologic arrangement would also prevent independent influence of one bridging structure on the dura and its contents as they are harmoniously integrated.

E12 sheet plastination also provided information regarding the intricately layered format of the upper cervical dura, which is now identified as being composed of soft-tissue components from multiple sources. From posterior to anterior, dura at the cerebrospinal junction consists of three layers of periosteum (posterior, inferior, and anterior) from the occiput, whereas the innermost layer is composed of cerebrospinal dura. Considering the periosteal component did not completely invest within dura until the level of C3, the bridging tissues identified in this study at the atlantooccipital and atlantoaxial levels added onto the posterior aspect of the dura. Therefore, the resulting components that make up the spinal dura at the level of C2 is composed of (from posterior to anterior) periosteum from the lamina of the axis, deep fasciae from the OCI and RCPma, periosteum from the inferior pole of the posterior arch of the atlas, deep fascia of the RCPmi, anterior periosteum from the posterior arch of the atlas, the three aforementioned layers of periosteum from the occiput, and spinal dura. All bridging structures that insert into the dura have a relationship with multiple soft-tissue components at various spinal levels, making this region extremely complex.

The ligamentum flavum at the atlantoaxial interspace has been negated within previous studies of the atlantoaxial space [8,23]. The results of this study reveal that the ligamentum flavum exists at this vertebral interspace, but is smaller in its lateral dimensions when compared with other vertebral levels. The smaller width permits fascial bundles of the RCPma and OCI to breach the epidural space. The ligamentum flavum at this level was also identified by E12 sheet plastination.

The RCPmi, RCPma, and OCI myodural structures have thus far been defined as fascial components of specific suboccipital muscles that insert into the posterior surface of the dura mater [1,7,16]. Results of this study further define these structures to include a portion of the dorsal meningovertebral ligaments of the upper cervical spine. This study identifies the unique nature of these upper cervical myodural bridging structures to include periosteum from their corresponding vertebrae and deep fasciae of the suboccipital muscles. The term "meningomyovertebral" ligaments would be the appropriate nomenclature for describing the structure following the merging of fascial components with the dorsal meningovertebral ligaments of the first two vertebrae in the epidural space. This proposed nomenclature would also allow for appropriate differentiation between the RCPmi, RCPma, and OCI myodural upper cervical stabilizing structures, which involve contractile tissue, from the dorsal meningovertebral ligaments reported throughout the length of the epidural space [9,18].

E12 sheet plastination was used in this study to provide a detailed analysis of soft-tissue structures *in situ*. In contrast to conventional dissection measures, sheet plastination allowed for complex features of the upper cervical region to be viewed under low-power magnification without compromising morphologic arrangement. A limitation of E12 sheet plastination is that the process may shrink soft-tissue structures such as muscle. However, this process permits separation of membranous planes and allows for delineation of fascial membranes and potential spaces. Therefore, this "limitation" inadvertently provided a credible method for identifying the components of the upper cervical epidural space, the PAO membrane, and the components of cervical dura in their *in situ* arrangement.

A study that has a larger sample size and uses statistical evaluation is warranted to clarify variations in the morphology and existence of soft-tissue structures within the general population. A follow-up study using confocal microscopy in conjunction with E12 sheet plastination would be the next step providing a clearer delineation of each layer within the upper cervical region.

Conclusions

This study used E12 sheet plastination to evaluate more accurately the tissue composition of the cervical epidural space. This complex region of neuromusculoskeletal anatomy may be of special interest to neurosurgeons, and should be acknowledged during neurosurgical procedures because inadvertent disruption may lead to dural laceration, resulting in postsurgical infection [20,33]. Considering these cervical epidural structures may be easily visualized with magnetic resonance imaging [23], it is important for the clinician to have a full understanding of epidural anatomy to avoid mistaking these ligaments for peridural adhesions. The findings in this study may also be implicated in the pathophysiology of certain neurogenic disorders, such as, but not limited to, cervicogenic cephalgia and Hirayama-type amyotrophy [9,17,29].

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