Meningeal relationships to the spinal nerves and rootlets: a gross, histological, and radiological study with application to intradural extramedullary spinal tumors

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Abstract

Introduction Juxtapositional tumors of the spinal nerve roots have been noted to not only interact with the roots at various vertebral levels, but also differ among patients. Therefore, the aim of the current study was to elucidate the potential for variation among the relationships of the meningeal layers at different nerve levels.

Methods In 20 unembalmed adult cadavers and five fetal specimens, the spinal nerve roots from the cervical, thoracic, and lumbar regions were harvested with their associated meningeal layers and subjected to microdissection, histological analysis, or radiological imaging using 9.4-T MRI.

Results As the nerve rootlets passed from the cord, they received their root sheath covering from the pia. After crossing the subarachnoid space to reach the apertures in the dura, they received two additional looser sheaths, an outer from the dura and an inner from the arachnoid. The pia mater always ended proximal to the arachnoid, and the pia and arachnoid layers extended more distally along the roots with caudal descent. Although the dorsal and ventral roots generally exited through separate dural openings, a single dural opening was also observed, often in the lower spinal regions. Thin intradural septations almost always separated the dorsal and ventral rootlets. The left and right sides frequently differed within individuals.

Conclusions On the basis of our study, variations of the meninges surrounding the spinal nerve roots are common, but themes do exist. Such data support surgical observations of the different interactions between tumors in these regions with surrounding neural tissues.

Keywords Spine · Spinal cord · Nerves · Roots · Meninges · Coverings · Anatomy · Tumor

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Introduction

Dorsal and ventral rootlets within the spinal subarachnoid space converge at the intervertebral foramen as a segmental spinal nerve [20]. As the rootlets approach the intervertebral foramen, their pial and arachnoidal layers merge with the dura

mater of the thecal sac; in this region, these meningeal layers fuse with the normal peripheral nerve coats. The three peripheral nerve connective tissue coverings are the epineurium, perineurium, and endoneurium. The epineurium is the most external. It is made up of dense, irregular, connective, and adipose tissues and binds nerve fascicles into a nerve trunk [18]. With multiple fascicles, the epineurial layer may be divided into epifascicular and interfascicular parts [22]. The perineurium consists of flat perineurial cells and collagen fibers and ensheathes nerve fibers into a fascicle [15]. The endoneurium consists of reticular and collagen fibers and an extracellular matrix occupying the space between the nerve fibers within the fascicle [11]. The endoneurium surrounds an axon and its Schwann cells [19].

Among intradural, extramedullary spinal tumors, 90 % are nerve sheath tumors (schwannoma, neurofibroma, ganglioneuroma) or meningiomas [12]. Such tumors near the spinal nerves behave differently not only at various vertebral levels, but also among patients and between sides in the same patient. For example, some tumors respect arachnoid layers, whereas others do not and can involve adjacent nerve tissue to the point that it must be sacrificed in order to remove the tumor fully. The present cadaveric study was performed in order to establish potential anatomical reasons for such observations.

Materials and methods

In 20 unembalmed adult cadavers (eight male, 12 female; age at death 43–101 years, mean 65 years) and five fetal specimens (two male, three female; age at death 10–14 weeks, mean 11.5 weeks), the spinal nerve roots and nerves from the cervical, thoracic, and lumbar regions were either dissected (Fig. 1) or harvested with their associated meningeal layers and subjected to histological examination and radiological imaging using a custom 9.4-T MRI (GE, CT). Specimens were obtained following laminectomy of the spine using an

Fig. 1 Example of the gross dissection of the intact spinal cord with meninges and lateral nerve sheaths in the thoracic region. Note the dorsal root ganglion within its dural sheath in the *upper left* of the photograph. Spinal nerves are also seen dividing into ventral and dorsal rami

osteotome or bone cutting oscillating saw (Stryker, Kalamazoo, Michigan). After the overlying laminae and spinous processes had been removed, the thecal sac and enclosed spinal cord and nerve rootlets were also removed, including the spinal nerve sheath 5 cm lateral to the intervertebral foramen. Of the 50 sides, 25 were chosen randomly for microdissection, 15 (seven left and eight right) for immunohistochemical examination, and 10 (five left and five right) for radiology. For gross analysis of specimens, ×2.5 surgical loupes and a surgical microscope (Zeiss, Germany) were used for dissection. For histochemistry, tissues were fixed in 10 % buffered formalin, embedded in paraffin, sectioned into 5-μm-thick sections (axial and longitudinal), and stained with hematoxylin and eosin, Verhoeff-Van Gieson, reticulin nuclear fast red, or Masson's trichrome. Images were captured with a microscope (Olympus, Center Valley, PA, USA) equipped with a camera.

For radiological specimens, sagittal, coronal, and axial slices at 5-μm thickness were cut serially through the samples. The axial images had an in-plane resolution of 59×59 μm and a slice thickness of 0.25–0.5 mm. The coronal plane had an in-plane resolution of 59×99 μm and a slice thickness of 0.25 mm.

Results

Gross observations

At all three levels (cervical, thoracic, and lumbar), each dorsal and ventral root had a separate dural covering as it exited the vertebral canal. There were some exceptions, especially in the thoracic region where the individual sheaths were so thin that both roots could share one dural sheath. Nevertheless, in the cervical region, the rootlets mostly exited in two separate and distinct dural sleeves from the thecal sac and there was no sheath holding these sleeves together. In the thoracic region, there was a thin sheath around the dura surrounding both the ventral and dorsal roots. This sheath was not always confluent with the spinal cord dura, as the two roots mostly exited the thecal sac in separate ostia; however, these two ostia were closer together than those in the cervical region. Each sheath was encased in a dense perineural sheath. The sheath surrounding the two roots now became a little thicker and often tapered as the roots exited the thecal sac. There were no obvious gross, histological, or radiological differences between the adult and fetal specimens.

Dural exit sites

Cervical levels Most often, the dorsal and ventral roots exited the dura through two separate ostia and were ensheathed in two separate dural tubes. Two dural openings were often found in the cervical region. The ventral root flowed into the

spinal nerve after the dorsal root ganglion, at which point, the two rootlets finally shared a common dura (Fig. 2). On occasion (15 %), both the ventral and dorsal roots exited out of a single ostium and shared a common dural sheath (Fig. 2).

Thoracic levels In the thoracic region, the dorsal and ventral roots exited through separate ostia in the dura, each having a separate dural sheath outside of the thecal sac (Fig. 2). They adhered loosely via a thin connective tissue sheath surrounding the individual dura. This connective tissue membrane also surrounded the dorsal root ganglia and was very thin.

Lumbar levels Generally, a single dural ostium was seen in the lumbar region (Fig. 2). However, a thin membrane was usually (>70 %) found inside these ostia and separated the rootlets as they exited. Outside the dural in this region and around the dorsal root ganglion was a thicker peridural membrane.

Histological observations

Histological sections revealed several consistent anatomical features of the meningeal layers. At the sites of attachment to the spinal cord, the rootlets were ensheathed in pia mater extending from the spinal cord. Generally, the rootlets were ensheathed in two layers of pia mater, described in detail below. The pia mater ended medial to the arachnoid, and the arachnoid ended medial to the dura mater. The pia and arachnoid layers extended more laterally along the nerve rootlets in the lumbar region and had the shortest extensions in the cervical region (Fig. 3a–c). Often, and proximal to the intervertebral foramen, the arachnoid included outer and inner layers. In the cervical region, the inner layer was frequently reticular (Fig. 4). An arachnoid septation divided the intraspinal dorsal and ventral rootlets. For the lumbar nerve roots, a common

arachnoid sheath usually enveloped the dorsal and ventral roots. Thin intradural septations were almost always found separating the dorsal and ventral rootlets.
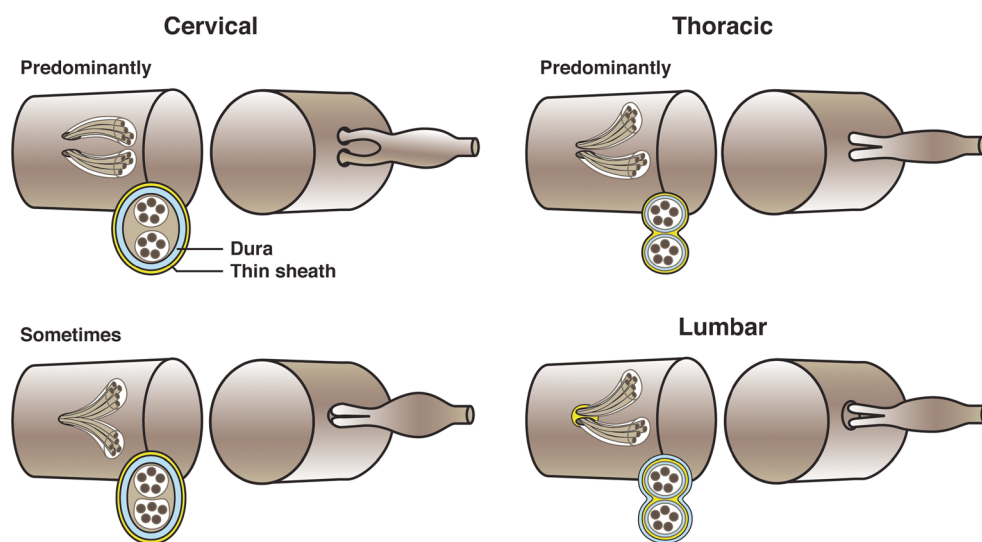
Fusion of the dura, arachnoid, and pia layers with their corresponding peripheral nerve coverings was not always distinct, but there were some anatomical patterns. In general, the pia-arachnoid became perineurium at the proximal pole of the dorsal root ganglion and at a comparable site on the ventral rootlets. These transitions tended to occur more laterally with descent along the spinal cord, and in some cases, the arachnoid to perineurium transition extended beyond the distal pole of the dorsal root ganglion. For example, all transitions occurred intraspinally in the cervical region and extraspinally in the lumbar region. We observed differences in transitions not only from cranial to caudal, but also from anterior to posterior. For example, the transitions of the dorsal roots were generally more lateral than those of the ventral roots.

Histological staining (hematoxylin and eosin for general connective tissues, Verhoeff-Van Gieson for elastin, reticulin nuclear fast red for reticular fibers, and Masson's trichrome for collagen) revealed that the pia mater comprised two layers, which frequently interdigitated. The inner pial layers were more robust and had occasional elastic fibers. The outer pial layer was made up of flattened meningeal cells, and reticulin fibers were often seen. Additionally, the outer layer was composed of interlaced bundles of connective tissue, which were mostly parallel and longitudinal. Both surfaces of this inner layer were covered with endothelial cells, and there was a network of fine elastic fibers near the basal surfaces of the endothelial cells.

Radiological observations

As a whole, the meningeal layers of the spinal nerves were difficult to differentiate on MRI, even at 9.4 T. Positionally, an

Fig. 2 Dural ostia/sheaths and relationships to exiting nerve roots in the **a** cervical (in 15 %, the cervical arrangement was varied as shown), **b** thoracic, and **c** lumbar regions. The *inset* drawings depict the arrangement of connective tissues immediately proximal to the dorsal root ganglion
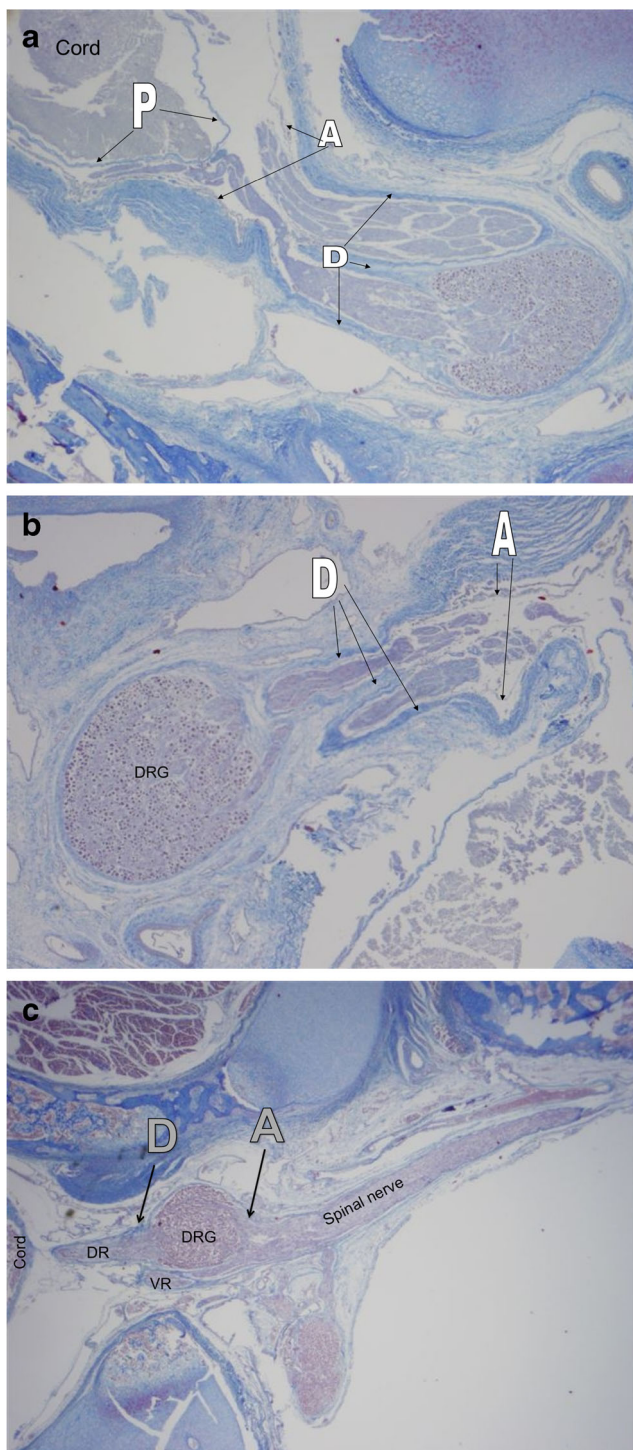
Fig. 3 Examples of histological (trichrome) sections through the **a** cervical (×4), **b** thoracic (×4), and **c** lumbar (×2) regions. *P* pia mater, *A* arachnoid mater, *D* dura mater, *DRG* dorsal root ganglion, *DR* dorsal root, *VR* ventral root
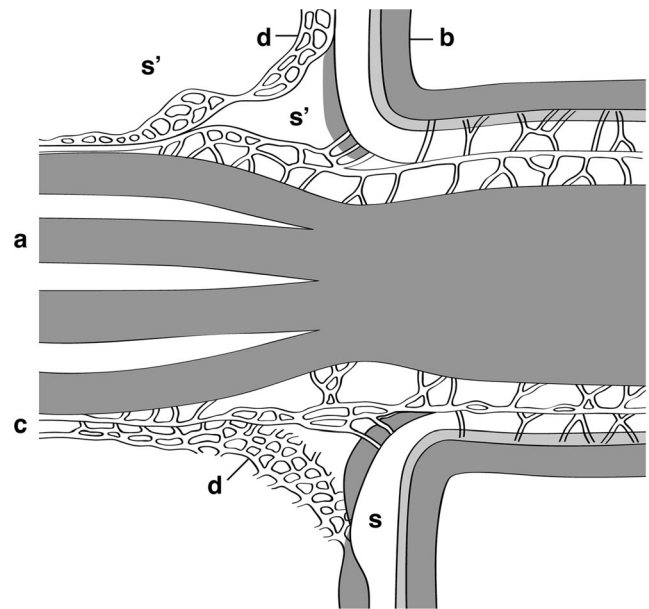

Fig. 4 Schematic longitudinal section: nerve root condensing into a common bundle (*a*), dura mater (*b*), arachnoid (*c*), reticular layer of arachnoid (*d*), subdural space (*s*), and subarachnoid space (*s'*)

seen on parasagittal images, although it was not possible to differentiate arachnoid from pia or to visualize lateral transitions into perineurium or endoneurium. However, as contrasted MRI studies can identify spinal vasculature, the orientation of the meningeal layer could be inferred.

Discussion

Vakili [23] described each spinal root/nerve complex as having five parts: subarachnoidal, subdural, extradural/intraspinal, intraforaminal, and extraforaminal juxtavertebral. The nerve roots, with their separable meningeal sheaths, pass into the dura. These sheaths unite peripheral to the dorsal root ganglion, subsequently forming the epineurium of the nerve. The nerve roots and the spinal nerves within their meningeal sheaths are attached through the dural layer to the periosteum at the margins of the intervertebral foramen. We found significant variation in the meningeal layers of the spinal nerve rootlets between sides at the same level, between ventral and dorsal rootlets on the same side, and at different regions of the spinal cord. However, themes did exist.

Frykholm [6] reported that each spinal nerve root enters a separate dural sheath and that each ostium is separated by a distinct interradicular septum. Furthermore, this author stated that each dorsal dural sheath can extend to cover the dorsal root ganglion, but normally the dorsal root ganglion is only covered over its medial half, and that the ventral sheath is, as a rule, patent further laterally. Frykholm [6] also stated that the arachnoid encloses each nerve root individually at its exit from the spinal cord and extends for a certain distance into the dural
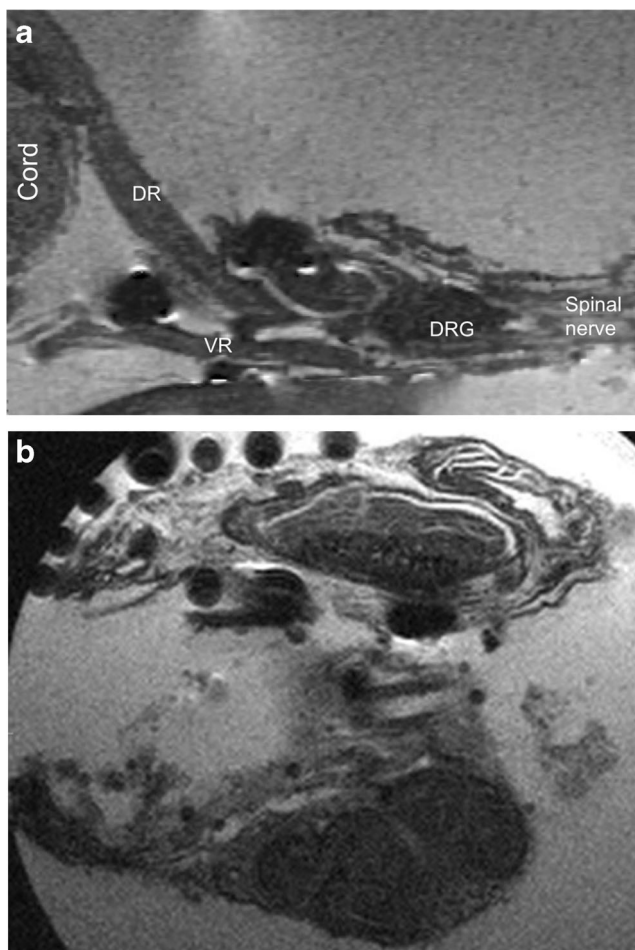
outermost layer (dura mater) could be appreciated on longitudinal sections at the intervertebral foramen and within the spinal canal (Fig. 5a, b). The transition of dura to epineurium was not clear on any of the MRI sequences. Proximal to the intervertebral foramen, layers deep to the dura mater could be

Fig. 5 Examples of MRI sections through the spinal rootlets and nerves: **a** axial and **b** parasagittal with dorsal root above and ventral root below. *DR* dorsal root, *VR* ventral root, *DRG* dorsal root ganglion

sheath. He found strictures in the dural sheath ostium in some patients and fibroblastic reaction and thickening/stenosis of these outlets, release of which resulted in immediate resolution of his patients' symptoms.

The inner portion of the dura mater lies adjacent to the arachnoid mater and consists of layers of cells called dural border, subdural, or neurothelial cells. These flattened cells are described as sinuous with interdigitating processes that create extracellular spaces with few intercellular junctions [24]. Dittmann et al. [4] found that the outermost layer of the dura consists mainly of collagen fibers. Stolinski [18] added to these data, specifying bundles of types I and III collagen fibrils and elastic fibers arranged in an undulating orientation. Vandenabeele et al. [24] found that the dura was composed of an outermost, loosely arranged fibroelastic layer, a middle fibrous portion, and an innermost cellular layer. This innermost layer was characterized by multiple interdigitating cell processes, a lack of extracellular collagen, significant extracellular spaces, and few cell junctions.

The outermost arachnoid layer, which had a reticular appearance in our study (Fig. 4), consists of tightly packed cells

with numerous tight junctions but no extracellular collagen [24]. The outermost arachnoid layer was always characterized by a distinct, continuous basal lamina on its inner surface toward the innermost collagenous portion of the arachnoid (arachnoid reticular cell layer). The interweaving arachnoid trabecular cells within this layer possessed numerous mitochondria and were anchored to the inner surface of the arachnoid barrier cell layer by desmosomes. The outer spinal arachnoid consists of flattened barrier cells that form an anatomical and functional barrier. Deep to the barrier cells is the arachnoid retinacular layer, which is composed of cells and collagen fibers. The innermost portion is the trabecular arachnoid layer, which consists of strands of densely packed thick collagen fiber bundles covered with arachnoid cells. Comparatively, Vandenabeele et al. [24] concluded that in view of its numerous tight junctions, the outermost arachnoid layer is an effective morphological and physiological meningeal barrier between the cerebrospinal fluid and the blood circulation in the dura. However, some have found that injections into the subperineural space of the peripheral nerve can spread into the subarachnoid space [2]. We also found that in some specimens, the subarachnoid space extended to the lateral pole of the dorsal root ganglion.

Schaeffer [16] described the roots of each nerve as receiving connective tissue support from the pia and arachnoid mater by passing through them. The basal laminae of the endoneurium, perineurium, and epineurium are confluent with the meninges. Specifically, the pia mater consists of one to two layers of flattened cells joined by desmosomes and gap junctions. The epineurium is generally described as continuous with the dura mater in the region of the dorsal root ganglion. Unlike the freely permeable epineurium, the perineurium is an effective barrier against, for example, anesthetics. This layer is composed of 3–15 concentric layers of flattened cells, which have a well-defined basement lamina. The internal zone consists of a single layer of perineurial cells connected by tight junctions. In the intermediate zone are multiple layers of perineurial cells. Between these cell layers in the external zone are types I and II collagen fibrils and elastic fibers arranged in circumferential, oblique, and longitudinal layers [7]. Layers of perineurial cells have a basal lamina of type IV collagen, laminin, fibronectin, and heparan sulfate [21]. The perineurium includes the connective tissue between perineurial cells as well as the perineurial cellular component [10, 11]. The endoneurium runs mostly longitudinally along the nerve fiber and is composed primarily of collagen bundles. This meshwork serves to support capillaries and connective tissue cells such as fibroblasts, mast cells, and macrophages [5].

Nittner [13] reported the incidence of intradural extramedullary tumors as 0.3 out of 100,000 people. Nittner also concluded that 50 % of these tumors are found in the thoracic spine and occur in the cervical and lumbosacral spines at 22 and 18 %, respectively [13]. Tumor types in this

region include nerve sheath tumors, e.g., schwannomas in 23–48 %, meningiomas in 9.6–35 %, neurofibromas in 4–23 %, and metastatic tumors in 6.4–25 % [3, 13, 17]. Imaging features can help to determine the site of origin of these various tumors in the intradural extramedullary region, including changes to the epidural fat and compression or widening of the subarachnoid space. For example, an epidural tumor replaces the epidural fat, displaces the dura, and compresses the subarachnoid space [9]. A subarachnoid tumor increases the subarachnoid space but generally does not affect the epidural fat.

Nerve sheath tumors most commonly arise from the dorsal rootlets and meningiomas from arachnoid cap cells in the dura mater near the nerve sleeve [1]. Seventy percent of nerve sheath tumors are intradural/extramedullary in location, 15 % are purely extradural, and a further 15 % have both intradural and extradural components ("dumbbell" lesions) [14]. Fewer than 1 % are intramedullary. Moreover, up to 45 % of tumors of the nerve roots occur in patients with neurofibromatosis [8]. Neurofibromas often encase nerve roots, but schwannomas more commonly displace adjacent nerve roots as they grow asymmetrically.

Anecdotally, surgeons and radiologists have found that the various nerve root tumors can have variable anatomic and functional expressions based on the level of the spinal cord and whether they involve the ventral or dorsal rootlets [1, 12]. Such inconstant findings might be due to the variable meningeal relationships to the nerve rootlets as found in our study. For example, an arachnoid septation divided the intraspinal dorsal and ventral rootlets at most levels. Such a meningeal interface could inhibit tumor growth at the levels where present but allow growth to continue at levels where absent. Also, tumors of the nerve roots of lumbar levels often involve both the dorsal and ventral rootlets and the lack of an interface at these levels is supported by our finding that a common arachnoid sheath enveloped both the dorsal and ventral lumbar nerve rootlets; i.e., there was no intervening arachnoid septation. Additionally, as seen in our study, the inner pial layer was thicker and occasionally contained elastic fibers at various levels. Such small histological differences could play a role in the way that intraspinal tumors behave.

Conclusions

On the basis of our study, variations of the meninges surrounding the spinal nerve roots are common, but themes do exist. Such data support surgical/radiological observations that the interactions between tumors of these regions and neural tissues differ among patients and between sides in the same patient.

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