Mechanism of action of the erector spinae plane block: distribution of dye in a porcine model

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ABSTRACT
This study aimed to describe the anatomical distribution of dye injected in the erector spinae plane (ESP) in a porcine living model, which could aid to reveal factors potentially relevant to the unexplained clinical effects of the ESP block. Six pigs received 0.6 mL/kg of 0.25% new methylene blue at the level of the sixth thoracic vertebra through either a cranial-to-caudal or a caudal-to-cranial in-plane ultrasound-guided bilateral ESP injection 20 min before euthanasia. Spread of dye evaluated through transverse cryosections (four injections) extended from T5 to T10 and from T5 to T8 when a cranial-to-caudal direction of injection was used, and from T5 to T9 and from T5 to T8 when the opposite direction of injection was used. A median of 4.5 medial and lateral branches of the dorsal rami was observed stained through anatomical dissection (eight injections), regardless of the direction of injection. No evidence of dye was found in the thoracic paravertebral or epidual spaces, where the dorsal root ganglia, ventral rami and rami communicantes are located. In all the cases, dye solution was found in the prevertebral thoracic lymph nodes.

In this study, ESP injection resulted in a median spread over five spinal segments (12 injections), staining the lateral and medial branches of the dorsal rami of the spinal nerves, regardless of the direction of the needle used.

INTRODUCTION
The erector spinae plane (ESP) block is an ultrasound (US)-guided interfascial regional anesthetic technique in which a local anesthetic is deposited deep to the erector spinae muscles. Single injections of a local anesthetic between the erector spinae muscles and the transverse process of a thoracic vertebra result in a predictable distribution of injectate mainly along the musculofascial plane deep to the erector spinae muscles. Previous studies suggest that part of the clinical effect of the ESP block is given by a theoretical anterior distribution of local anesthetic through the intertransverse connective tissue or the costotransverse foramen to bathe the ventral rami of the spinal nerves, dorsal root ganglia (DRG) and sympathetic chain. However, to date, most studies are not able to unambiguously determine the path of injectate to explain the mechanism of action of the ESP block.

The disposition of the epaxial and hypaxial muscles and their associated innervation pattern are differentiated from early embryological stages of development. The paraspinal muscles are wrapped by layers of the thoracolumbar fascia and dense intertransverse connective tissue, which may constitute a physical barrier that limits the connection path between epaxial and hypaxial muscle compartments, mainly traversed by nerves, blood and lymphatic vessels. This structured organization of the aforementioned compartments and their limited connecting pathways call for a thorough revision of the to date proposed mechanism of action of the ESP block.

In pigs, the anatomical disposition of the musculature (ie, transversospinals and erector spinae muscle complexes), nerves, and lymphatic and blood vessels contained in the lateral vertebral groove is similar to that in humans, making the porcine a representative model to investigate the distribution pattern of dye after an ESP injection.

Additionally, the use of a living model could aid to reveal factors potentially relevant to the unexplained clinical effects of the ESP block. The objective of this experimental study was to describe the anatomical distribution of 0.6 mL/kg of 0.25% new methylene blue through either a cranial-to-caudal or a caudal-to-cranial in-plane US-guided ESP injection 20 min prior to euthanasia in a porcine model.

METHODS
Six young and healthy pigs weighing 28±2.1 kg that were part of an unrelated project that required general anesthesia and euthanasia were enrolled in this study.

ESP US-guided injection
All pigs were under stable general anesthesia during ESP US scanning and injections, which were performed by a veterinary anesthesiologist experienced in US-guided regional anesthesia. A portable US machine (M-Turbo, SonoSite, Washington, USA) with a 6–13 MHz, 45 mm wide linear array transducer (HLF 38, SonoSite) was used to guide all injections. A parasagittal US scan over the tip of the left and right transverse processes of the sixth thoracic vertebra (T6) was performed in order to identify the erector spinae muscle complex, the erector spinae interfascial plane and the transverse processes and the paravertebral space (PVS). The right and left sides were randomly assigned to receive either a cranial-to-caudal or a caudal-to-cranial in-plane US-guided ESP injection at the level of T6 (12 injections). A 21-gauge 100 mm sonographic needle (Ultraplex 360; B. Braun Medical,
Pennsylvania, USA) was introduced and advanced in-plane through the epaxial muscles until its tip contacted the lateral edge of the transverse process of T6. After negative aspiration, a total volume of 0.6 mL/kg of 0.25% new methylene blue was injected while observing spread of the solution in the erector spinae interfascial plane. Injections were performed only if no resistance to injection (<15 psi) was perceived. Additionally, signs of injection in the thoracic PVS, such as hypaxial muscle enlargement or parietal pleura ventral displacement, were recorded. The same procedure was repeated in the contralateral side, with needle insertion and advancement in the opposite direction.

**Anatomical study**

All animals were euthanized 20 min after the second injection for reasons unrelated to this study and were frozen at −20°C for a minimum period of 72 hours. Two randomly assigned animals were kept frozen to perform transverse cryosections, and the rest were thawed before anatomical dissection in order to determine the distribution of dye. Anatomical evaluations were performed by a blind veterinary anatomist.

In the frozen cadavers assigned to cross-sectional evaluation, 2 cm transverse slices were obtained from the 2nd thoracic vertebra to the 12th thoracic vertebra (T12), followed by a delicate dissection to investigate the spread pattern of dye and a potential path between the epaxial and hypaxial compartments.

In the thawed cadavers, dissection of the hypaxial and epaxial compartments was performed through different approaches to avoid unintentional contamination of the spinal nerves and thoracic PVS with dye. In order to reach the hypaxial compartment, the posterolateral body wall was resected and carefully lifted, and the thoracic organs were gently removed while noting the possible presence of dye. The parietal pleura and endothoracic fascia were reflected in order to assess the presence of dye staining the sympathetic chain, the ventral rami of the spinal nerves and the intercostal nerves. Then, the dorsal midline was incised and reflected laterally, and the superficial epaxial muscles were removed to expose the thoracolumbar fascia, which was then detached from its attachment on the spinous processes and reflected laterally. The intermuscular plane between the longissimus thoracis and multifidus muscles was carefully separated and inspected in order to evaluate the presence of dye on the medial branches of the dorsal rami of the spinal nerve. The intermuscular fascial plane between the longissimus thoracis and iliocostalis thoracis muscles was carefully inspected to determine the number of lateral branches of the dorsal rami of the spinal nerve stained. The extent of the cephalocaudal spread of dye in the ESP was also noted. Nerves were considered successfully stained when the dye solution was distributed around their circumference for a length of >1 cm. The stained nerve branches were followed toward the lateral vertebral foramen to evaluate the origin of the dorsal rami of the spinal nerves. Finally, a laminectomy was performed in order to evaluate the presence of dye inside the epidural space and in the vicinity of the DRG. Numerical variables were expressed as median (range).

**RESULTS**

In all the animals, the sonoanatomy of the lateral vertebral groove and its structures were easily recognized and the sixth thoracic transverse process was identified (figure 1). During all injections, a craniocaudal hydrodissection effect of the injectate was observed in real time in the erector spinae interfascial plane.

During none of the injections were signs of injectate spreading toward the hypaxial muscle compartment or the PVS observed. Anatomical evaluation of the transverse cryosections (four injections) showed that the dye solution spread on the ventromedial aspect of the longissimus thoracis muscle from T5 to T10 and from T5 to T8 when a cranial-to-caudal direction of injection was used and from T5 to T9 and from T5 to T8 when the opposite direction of injection was used. On both sides, spread of dye followed mainly two paths: a medial path between the longissimus thoracis and multifidus muscles, bathing the medial branches of the dorsal rami, and a lateral path between the longissimus thoracis, the levatores costarum and iliocostalis thoracis muscles, bathing the lateral branches of the dorsal rami (figure 2). Dye was also observed in the prevertebral lymph nodes (lymphonodi thoracici aortici). In these cadavers, there was no evidence of dye on the ventral rami of the spinal nerves, intercostal nerves, the thoracic paravertebral or epidural spaces.

Gross anatomical dissection (eight injections) allowed observation of the spread of dye solution along the connective tissue of the intertransverse spaces and along the ventromedial aspect of the longissimus thoracis muscle, adjacent to the levatores costarum, iliocostalis thoracis, multifidus and spinalis muscles (figure 3). ESP injection successfully stained a median of 4.5 (range 4–6) and 4.5 (range 4–7) medial and lateral branches of the dorsal rami, when cranial-to-caudal and caudal-to-cranial directions of injection were used, respectively. Within each treatment, the cephalocaudal spread of dye along the ESP coincided with the segments in which the branches of the dorsal rami were affected. The most cranial and caudal branches of the dorsal rami affected by the ESP injection were T3 and T10, respectively (figure 4). Only in one case stain was found in the dorsal branch, before its ramification, in coincidence with the injection site. In all the cases, lateral spread of dye extended between the longissimus and iliocostalis muscles, and between these and the external fasciae of the external intercostal muscle, consistently bathing the lateral branch of the dorsal rami up to the limit imposed by the thoracolumbar fascia (TLF). Spread of dye beyond the TLF did not occur.

In all the cases, dye solution was found in the prevertebral thoracic lymph nodes. In 9 of 12 injections, the lymphonodi thoracici aortici were highly stained (figures 2 and 3). The lymphonodus mediastinales craniales and lymphonodus
mediastinales caudales were slightly stained in one and two injections, respectively. In one injection, dye was found in lymphatic vessels associated with the lymphonodus mediastinales craniales.

No evidence of dye was found in the thoracic paravertebral or epidural spaces (figure 6). In none of the cases were the DRG, ventral rami, rami communicantes and sympathetic trunk stained (figure 7).

DISCUSSION
This is the first study in which dye was injected in the ESP in living animals and then its spread evaluated postmortem. The main findings of this study suggest that the injection of 0.6 mL/kg of dye injected in the ESP at the level of the T6 results in an extensive spread over a median of five spinal segments, staining the target lateral and medial branches of the dorsal rami of the spinal nerves, regardless of the direction of the needle used. In contrast, dye was not found in the epidural or PVSs, where the ventral rami of the spinal nerves and the rami communicantes

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spinal musculature and the intertransverse connective tissue seemed to hinder the passage of dye into the PVS, which is a similar finding to most cadaveric human studies.\textsuperscript{3,6} Similarly, a recent study performed in nine canine cadavers receiving thoracic ESP injections failed to identify by gross anatomical dissection, MRI and transverse cryosection the presence of injectate in the thoracic PVS or a communicating path between the epaxial and hypaxial compartments.\textsuperscript{19}

Lateral spread of dye along the musculofascial plane between the longissimus and the levatores costarum, external intercostal and iliocostalis muscles did not extend beyond the lateral limits of the thoracolumbar fascia, regardless of the anatomical model of the TLF considered (two layers vs three layers). This finding is in agreement with most human cadaveric studies\textsuperscript{3,6} However, previous human studies bear a segmental incongruence in which the presence of dye on muscle surface is not always accompanied by compromise of the correspondent dorsal rami.\textsuperscript{3,5,18} For example, Ivanusic \textit{et al} reports a distribution pattern from T1 to T12 in which only 3/12 segments the dorsal rami were stained in more than 50% of the injections.\textsuperscript{2} In the pigs used in this study, the interfascial distribution of dye coincided with the correspondent segmental branches of the dorsal rami, and therefore, results are similar to the reported number of dorsal rami affected in human studies.\textsuperscript{3,5,18}

Stain did not spread beyond the limits of the lateral vertebral foramen, and anterior spread of dye into the PVS did not occur. Human cadaveric studies that report anterior spread of dye into the PVS find only irregular traces of stain reaching the surface of the ventral rami without detailing the pattern and extension of nerve involvement.\textsuperscript{3,5,18} Therefore, the apparent little correspondence between the extensive spread of dye commonly found in the epaxial compartment and its minimal presence in the PVS,\textsuperscript{1,3,6,18} together with an uncertain spread of dye to be considered as a nerve block,\textsuperscript{20} challenges the theory of the ventral rami being involved in the clinical effect of the ESP block. Although dye leakage into the hypaxial compartment during dissection should be considered as a factor that may contribute with anterior presence of dye, in this study, this can be discarded because the dissection of the PVS was performed before dissecting the epaxial compartment.

Pressure exerted during injection of a large volume of dye in the ESP has also been postulated to favor its spread into the PVS.\textsuperscript{5,21} However, the pressure exerted during injection should theoretically be ‘consumed’ in the path of least resistance favoring fluid toward the ESP,\textsuperscript{22} without forcing its passage through the anatomical barrier existing between the epaxial and hypaxial muscle compartments,\textsuperscript{11} regardless of the volume injected.\textsuperscript{23} In this study, neither the pressure exerted nor the injection of a large volume (equivalent to 40 mL in a 70 kg human) produced real-time US-visible changes in the PVS compatible with anterior spread of dye.

The presence of dye staining prevertebral thoracic lymph nodes was observed in all animals. Such an intimate anatomical relation exists between lymphatic and nerve structures that the posterior intercostal lymph nodes are located close to the DRG and spinal nerves and the prevertebral lymph nodes near the sympathetic chain all along the thoracic vertebral column (figure 8).\textsuperscript{3,9} Some studies rely on MRI or CT to confirm the presence of injectate in the PVS, assuming that this indicates potential block of the ventral rami and sympathetic chain.\textsuperscript{3,9} However, none of these studies considered the possibility that

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**Figure 6** Ventral (anterior) view (A) and schematic representation (B) of the paravertebral space at the level of the sixth thoracic vertebra. The needle is pointing at the emergence of the ventral ramus from the spinal canal. Cd, caudal; Cr, cranial; L, lateral; M, medial.

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**Figure 7** Dorsal (posterior) view of the spine after the epaxial musculature was removed and a laminectomy was performed. Note the spread of dye over the external facia of the external intercostal muscles. Insert: scheme for anatomical references. T5, fifth thoracic vertebra; T8, eighth thoracic vertebra.
Original research

![Diagram of Lymph Drainage from the Thoracic Region]

**Figure 8** Lymph drainage from the thoracic region. Adapted from Rickenbacher et al. 

a contrast medium may be located inside lymphatic structures. Although in healthy conditions lymphatic vessels are not identifiable through MRI, pathological situations that course with lymphedema, such as chronic pain or cancer, may render the precise location of the injectate. Then, it appears reasonable to consider the possibility that the dye evidenced in the projection area of the PVS through imaging studies is located inside lymphatic drainage and not necessarily around nerves. This could be the rationale behind the inconsistent results between clinical and cadaveric studies in which lymphatic drain is absent.

Interestingly, current neuroimmunological research is able to provide compelling evidence for an interaction between the immune and the nervous systems. It is known that the immune system has a multidimensional and critical role in pain and inflammation through an active crosstalk with nociceptor neurons, in which both the primary sensory fibers and the sympathetic noradrenergic innervation of the lymph nodes are responsible for the neuropeptide release (eg, substance P, calcitonin gene-related peptide) and for the lymphatic drain flow, respectively.

In fact, it is known that the application of capsaicin inside lymph nodes desensitizes sensory fibers attenuating the neurogenic inflammatory response and the associated pain.

Therefore, the possibility that the anesthetic draining from the ESP in sufficient volume and concentration to desensitize sensory nerve fibers contiguous to lymphoid parenchyma is an unconventional but attractive theory that warrants further investigation.

Although a less convincing but plausible theory, the inherent permeability of the thin lymphatic vessels that pass through the PVS and the possible extravasation of traces of local anesthetic toward the DRG may affect nociception and may partially explain the clinical effects of the ESP block that are unrelated to the desensitization of the dorsal rami. In fact, the increased permeability of the lymphatic vessels in inflammatory states may amplify the clinical effect of the ESP block reported in patients with chronic pain.

There are some major limitations to this study. First, we used a porcine model to represent spread of local anesthetic into the back and makes the porcine a representative model for human studies. Second, the sample size was limited to the available animals enrolled for other studies and did not allow drawing of statistically significant conclusions. Third, euthanasia was performed 20 min after injection, and therefore, the model used was not able to demonstrate potential distribution in a living subject beyond that time. Lastly, the finding of dye in lymphatic structures draining from the ESP was unexpected. Although the pig lacks posterior intercostal lymph nodes, this observation calls for further studies specifically designed to investigate its potential relevance in the distribution of injectate and the clinical effect observed when performing ESP injections in the living.

**CONCLUSIONS**

In conclusion, this study demonstrated that the spread of the solution injected in the ESP was confined to the epaxial muscle compartment involving the lateral and medial branches of the dorsal rami regardless of the direction of the needle. No evidence of anterior spread of dye involving nerve structures, which could explain the reported clinical effect of the ESP block, was observed. A novel finding was the presence of dye in the thoracic lymph nodes, which demonstrates its migration from the ESP through its lymph drainage. This warrants further investigation in order to elucidate an additional mechanism of action involved in the clinical effect of the ESP block.

**Contributors** PEO: study design, injections, data collection, analysis of the results, manuscript preparation and artwork; SEF: study design, data collection, analysis of the results and manuscript preparation; PCR: dissections and data collection; NV: analysis of the results and manuscript preparation; CB: dissections, data collection and analysis of the results; DAP: study design, analysis of the results, manuscript preparation and artwork.

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