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Anatomic basis of the PENG block: dissection, cryo-cross-sections, and histology challenge a pericapsular target

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ABSTRACT

Background The pericapsular nerve group (PENG) block was developed to result in local anesthetic (LA) spread in the plane between the iliopubic ramus and the iliacus muscle. We aimed to investigate the anatomical and histological basis of injectate spread following a PENG block.

Methods We performed a PENG block in 10 cryopreserved cadavers under ultrasound guidance. A 22-gage, 50 mm needle was inserted using an in-plane approach. After contacting the bone at the target site, 20 mL of the solution mixed with the marker (methylene blue or heparinized erythrocytes) was injected. The dispersion of the injectate was analyzed using anatomical dissection, cryo-cross-sections, and microscopy.

Results Gross anatomical dissection demonstrated methylene blue staining in close proximity to the femoral nerve and its branches, without staining of the obturator nerve. Cryo-cross-sectional and histological analyses showed predominantly subepimysial and intramuscular localization of the injectate within the iliopsoas muscle compartment, without consistent involvement of the hip capsule. Histology identified small nerve branches within adipose-containing connective tissue planes.

Conclusions The injectate following a PENG block was predominantly confined to the iliopsoas muscle compartment. The presence of small intramuscular nerve branches suggests that intramuscular neural blockade may contribute to the analgesic effect. Potential femoral nerve involvement warrants further investigation.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The pericapsular nerve group (PENG) block provides effective analgesia after hip surgery and trauma, but its mechanism of action remains uncertain. Previous imaging and clinical studies have suggested predominant spread into the iliopsoas muscle compartment, with possible involvement of the femoral nerve.

WHAT THIS STUDY ADDS

⇒ Using dissection, cryo-cross-section, and histology, this study demonstrates that injectate following a PENG block is distributed predominantly within the iliacus and psoas muscles rather than within a distinct pericapsular or musculo-osseous fascial plane. Histology identified small intramuscular nerve branches, supporting intramuscular neural blockade as a potential mechanism of analgesia.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This multimodal methodology provides new insights into the tissue architecture at the PENG injection site and highlights important characteristics of musculo-osseous tissue interfaces that may influence local anesthetic spread. These findings may contribute to a better understanding of the variability and conflicting anatomical and clinical findings reported with fascial plane blocks.

INTRODUCTION

The pericapsular nerve group (PENG) block was introduced as a motor-sparing technique for pain relief in hip surgery.[1] It aims to block the articular branches of the femoral, obturator, and accessory obturator nerves that supply the anterior capsule of the hip joint, with the injectate presumed to spread in the plane between the iliopubic ramus and the iliacus muscle. In our recent patient study using three-dimensional CT, we did not observe injectate distribution within this plane.[2] Instead, the injectate was mainly located within the iliacus muscle. These findings suggested a potential risk of local anesthetic (LA) spread toward the femoral nerve, which may cause quadriceps weakness and increased risk of falls. This work generated significant discussion and letters to the editor,[3 4] prompting further investigation. In the current study, we combined gross anatomical dissection, cryo-cross-sections, and histological analyses in human cadavers to better define the anatomical basis and injectate distribution of the PENG block.

METHODS

Cadaveric material was handled in full compliance with regional regulations in Catalonia (Decree 294/1998), which govern the use of human cadavers for teaching, study, and research purposes. All dissections and macroscopic images were performed at the Surgical Neuroanatomy

Laboratory, Department of Human Anatomy and Embryology, Faculty of Medicine and Health Sciences, University of Barcelona (XS-B). Histological preparation and analyses were conducted at the Institute of Applied Molecular Medicine, CEU-San Pablo University School of Medicine, Madrid (MAR). A total of 10 cadaveric specimens (15 hips) were included. Three specimens (three hips) underwent gross anatomical dissection; five specimens (eight hips) underwent cryo-cross-sectioning; and two specimens (four hips) were used for histological analysis.

Ultrasound-guided PENG block

Following the originally described technique,[1] PENG blocks were performed using an M-Turbo ultrasound machine (Sonosite, Bothell, Washington, USA) equipped with a 6–13 MHz high-frequency linear transducer. A 22-gage, 30° bevel, 50 mm needle (Stimuplex D, B. Braun, Melsungen, Germany) was inserted using an in-plane approach from lateral to medial to reach the plane immediately lateral to the psoas tendon. After contacting the bone at the target site, the injectate was administered. In specimens designated for gross anatomical dissection and cryo-cross-sectioning (11 hips), 20 mL of 0.2% ropivacaine mixed with 0.01% methylene blue was injected to allow macroscopical visualization of the spread. In specimens for histological analysis (four hips), two hips received a 20 mL injectate consisting of 10 mL of 0.2% ropivacaine mixed with 0.01% methylene blue and 10 mL of heparinized erythrocyte solution. The heparinized erythrocytes served as a histological marker of injectate localization.[5] The remaining two hips were not injected and served as controls. All ultrasound images obtained during the PENG injections were recorded and archived for reference. The PENG blocks were performed by experienced anesthesiologists (PG and XS-B).

Gross anatomical dissection

Dissections were performed in sequential anatomical planes to identify key structures relevant to the PENG block, including the iliacus and psoas muscles, and the femoral and obturator nerves. Serial photographs were taken to document the distribution of the injectate relative to these structures. After removing the skin and subcutaneous tissue, the fascia lata was incised to expose the femoral triangle. The femoral nerve was identified lateral to the femoral vessels, while the obturator nerve was traced medially, emerging from the obturator canal. Deeper dissection exposed the psoas major and iliacus muscles, which converge to form the iliopsoas tendon, inserting toward the lesser trochanter. The iliopsoas was gently retracted to expose the anterior hip capsule and the femoral head–neck junction. This approach allowed direct visualization of the anatomical relationships relevant to the PENG block target area.

Cryo-cross-sections

Specimens were frozen and sectioned into 3 cm axial slices extending from the mid-pelvis to the level of the lesser trochanter. The distribution of methylene blue was systematically assessed at predefined anatomical landmarks, including the pelvis, obturator canal, obturator nerve, distal femoral nerve, and iliac fossa. Both intramuscular spread within the iliopsoas muscle and spread in the plane between the iliacus muscle and the iliac bone were evaluated at five predefined levels: (1)the anterior superior iliac spine (ASIS), (2) the anterior inferior iliac spine (AIIS), (3) the psoas tendon at the pelvic brim, (4) the acetabulum and the level of the lesser trochanter and (5) the hip capsule.

Histological analysis

Histological evaluation was performed to assess the injectate distribution at the target site of the PENG block, namely the enthesis of the iliacus muscle—the site where the iliacus muscle attaches to the bone. Two hips were injected with human heparinized erythrocytes, used as a particulate marker to evaluate early fluid spread within the tissue.[5] The remaining two hips were not injected and served as controls. After injection, the tissues were dissected into blocks and immersed in 10% buffered formaldehyde solution for 4 weeks. Histological analysis was conducted by an independent researcher (MAR) who was blinded to the injection status. The tissue blocks were sectioned into 2.5 mm slices perpendicular to the hip axis. The slices were then processed with paraffin wax and serially sectioned (5 slices of 3 µm thickness, at 20 µm intervals) on a microtome. Sections were stained with H&E under standard conditions and examined under a light microscope (Leica DM5500 B, Leica Microsystems, Wetzlar, Germany) at magnifications ranging from × 8 to ×800. Digital images were captured using a Leica DFC425 camera (Leica SCN Microsystems), and complete cryo-cross-sections were scanned using a Leica SCN400 slide scanner (Meyer Instruments, Houston, Texas, USA) and a Leica SCN Microsystems. The original images (gross anatomical dissection, cryo-cross-sections, and microscopic images) were digitally processed for clarity by MAR using Adobe Photoshop CC 2017.0.1 (Adobe Systems, San Jose, California, USA).

RESULTS

Anatomical and histological findings provided complementary insights contributing to the overall results and conclusions. Methylene blue was used as the marker for gross anatomical dissection (figures 1–3) and cryo-cross-sectional analysis (figure 4), whereas heparinized erythrocytes served as a histological marker in the microscopic analyses (figures 5 and 6).

Gross anatomical dissection

Findings were consistent across the three dissected hips. Methylene blue was observed in close contact with the femoral nerve and several of its branches along the anterior surface of the iliopsoas muscle, with distal spread limited to the level of the origin of the deep femoral artery (figures 1A,B, 2B and 3A).

No dye was observed at the level of the obturator nerve within the obturator canal, nor around the lateral cutaneous nerve of the thigh at the level of the ASIS (figure 2A,C).

Methylene blue was observed at the level of the anterior femoral head-neck junction and adjacent acetabular region (figure 3B).

Cross-section on cryopreserved tissue

Cryo-cross-sections were performed on eight hips from five cryopreserved cadavers to assess methylene blue distribution following PENG injection. Findings were consistent across all specimens (figure 4A–D; table 1). Distribution was evaluated at five predefined anatomical levels.

1. At the level of the ASIS and (2) AIIS, methylene blue was observed along the deep surface of the iliacus muscle, adjacent to the iliac bone, with additional staining extending into the muscle (figure 4A,B).
2. At the level of the psoas tendon at the pelvic brim, methylene blue was observed along the deep aspect of the iliopsoas muscle, beneath the iliopsoas tendon and extending to its medial aspect, while remaining enclosed within the iliopsoas fascia (figure 4B).

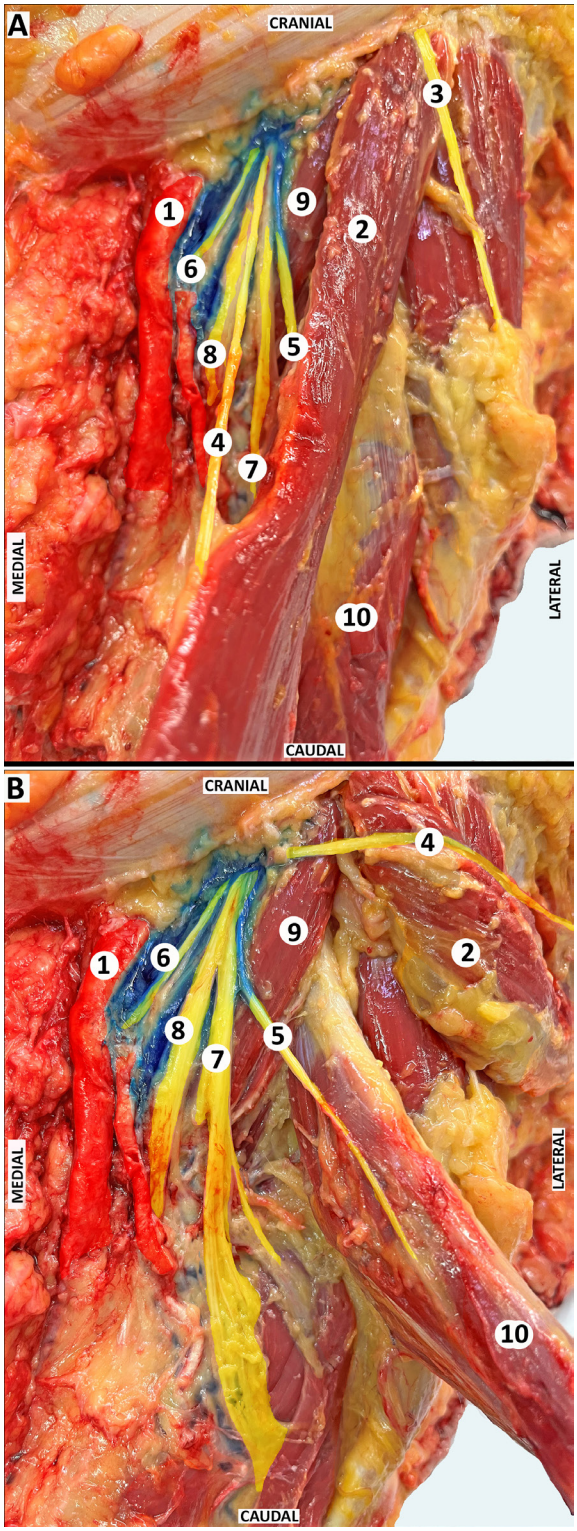

Figure 1 Femoral nerve distribution following a PENG block. (A) Initial femoral nerve dissection. (B) Dissection after displacement of the sartorius and rectus femoris muscle. 1, Femoral artery; 2, Sartorius muscle; 3, Lateral femoral cutaneous nerve of the thigh; 4, Nerve to the sartorius muscle; 5, Nerve to the rectus femoris muscle; 6, Nerve to pectineus muscle; 7, Nerve to the vastus lateralis and vastus intermedius muscles; 8, Nerve to the vastus medialis, nerve to the vastus intermedius and saphenous nerve; 9, Iliacus muscle; and 10, Rectus femoris muscle (displacedmoving from A to B). Blue staining indicates injectate spread. Colors were assigned to the various structures for clarity using Adobe Photoshop (Adobe, San Jose, California, USA).

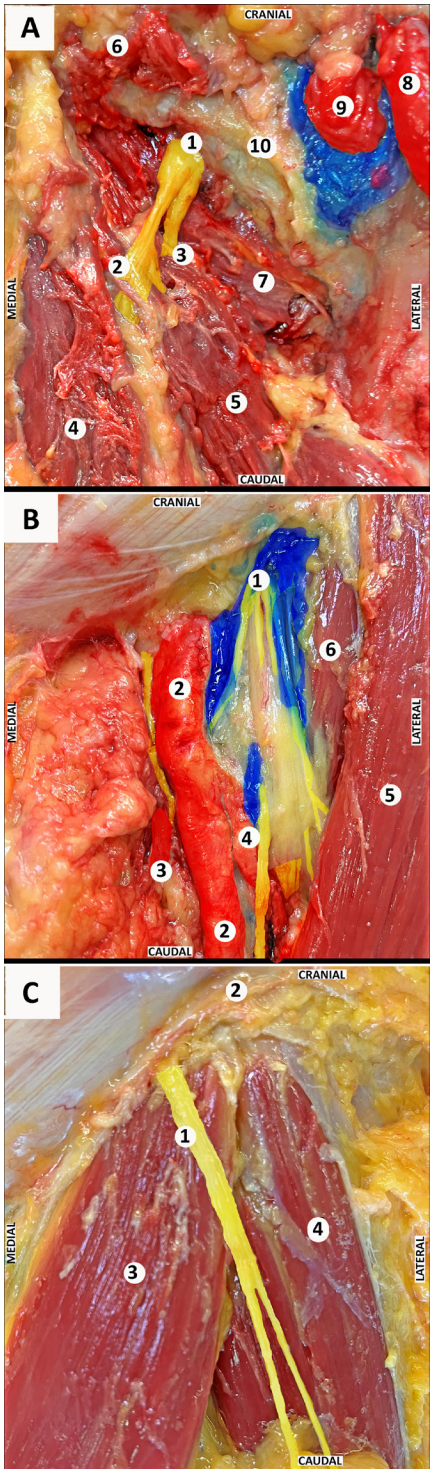

Figure 2 Injectate spread within the lumbar plexus nerves following a PENG block: dissection. (A) Obturator nerve. 1, Obturator nerve; 2, Anterior branch; 3, Posterior branch; 4, Adductor longus muscle; 5, Adductor brevis muscle; 6, Pectineus muscle (reflected): Origin of the pectineus muscle (cut); 7, Obturator externus muscle; 8, Femoral artery; 9, Femoral vein; 10, Superior pubic ramus, medial to the iliopubic eminence. (B) Femoral nerve. 1, Femoral nerve; 2, Femoral artery; 3, Femoral vein; 4, Deep femoral artery; 5, Sartorius muscle; 6, Iliacus muscle. (C) Lateral cutaneous nerve of the thigh. 1, Lateral cutaneous nerve of the thigh; 2, Anterior superior iliac spine; 3, Sartorius muscle; 4, Tensor of fascia lata muscle. Blue staining indicates injectate spread. Colors were assigned to the various structures for clarity using Adobe Photoshop (Adobe, San Jose, California, USA).

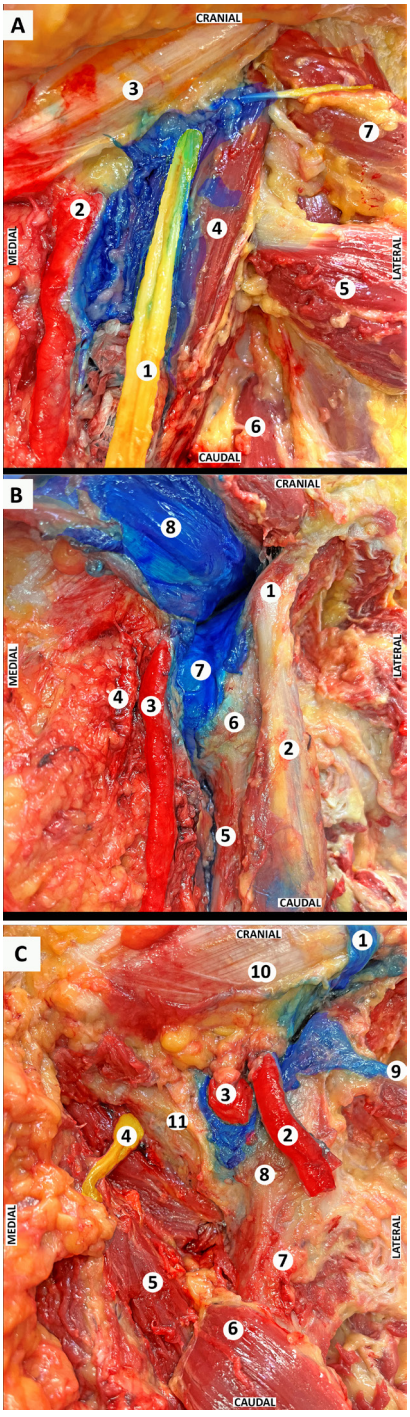

Figure 3 Injectate spread following a PENG block: dissection. (A) Femoral nerve spread. 1, Femoral nerve; 2, Femoral artery; 3, Inguinal area (abdominal wall); 4, Iliopsoas muscle; 5, Rectus femoris muscle (rejected); 6, Vastus lateralis muscle; 7, Sartorius muscle (reflected). (B) Psoas iliac spread. 1, Anterior inferior iliac spine; 2, Rectus femoris muscle; 3, Femoral artery; 4, Femoral vein; 5, Femur; 6, Head of the femur (anterior capsule); 7, Acetabulum; 8, Iliopsoas muscle (retracted). (C) Acetabulum spread. 1, Femoral nerve (displaced); 2, Femoral artery; 3, Femoral vein; 4, Obturator nerve; 5, Adductor longus muscle; 6, Pectineus muscle (displaced); 7, Femur bone; 8, Neck-head of the femur; 9, tendon of the rectus femoris muscle (displaced); 10, inguinal area (abdominal wall); 11, Superior pubic ramus, medial to the iliopubic eminence. Blue staining indicates injectate spread. Colors were assigned to the various structures for clarity using Adobe Photoshop (Adobe, San Jose, California, USA).

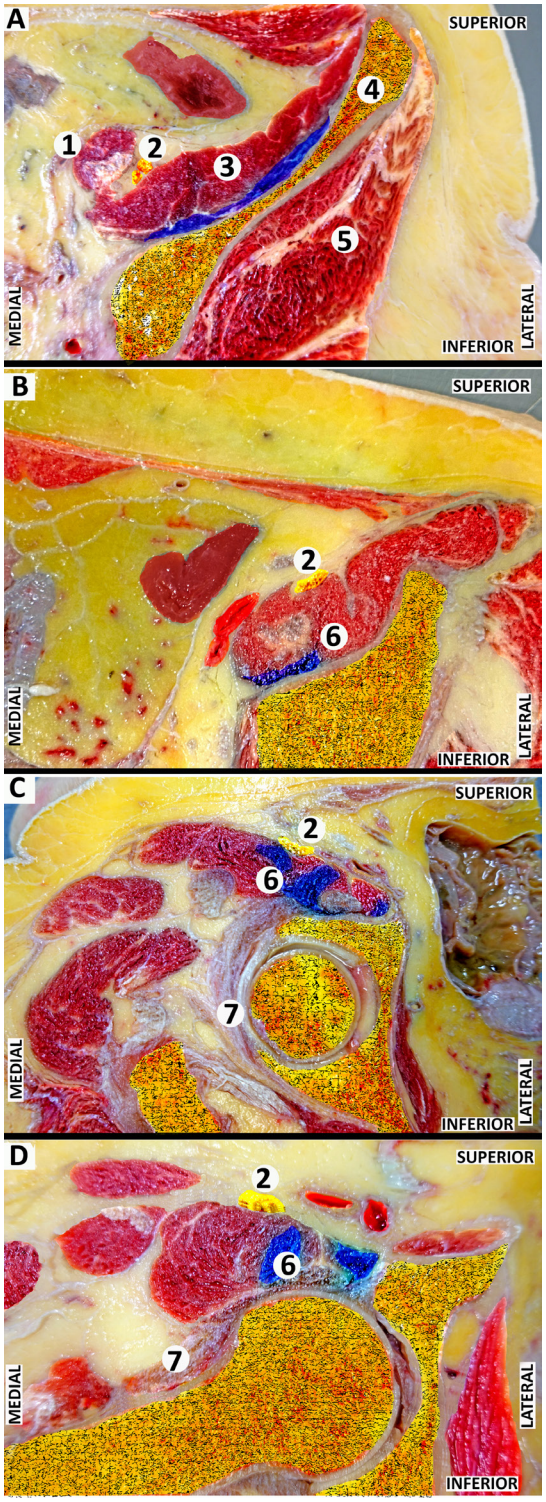

Figure 4 Injectate distribution following a PENG block: cryo-cross-sectional analysis. Sequential sections are shown. (A) At the level of the iliac fossa: preferential cephalad spread within the iliacus muscle. (B) At the level of the injection site posterior to the psoas major tendon: injectate located along the iliac bone near the pelvic brim. (C) At the level of the femoral head: staining confined to the iliopsoas muscle, with no capsular involvement. (D) At the level of the femoral neck: a similar distribution pattern, without evidence of capsular spread. 1, Psoas major; 2, Femoral nerve; 3, Iliacus; 4, Iliac bone; 5, Gluteal muscles; 6, Iliopsoas; 7, Hip capsule. Blue staining indicates injectate spread. Colors were digitally enhanced for clarity using Adobe Photoshop (Adobe, San Jose, California, USA).

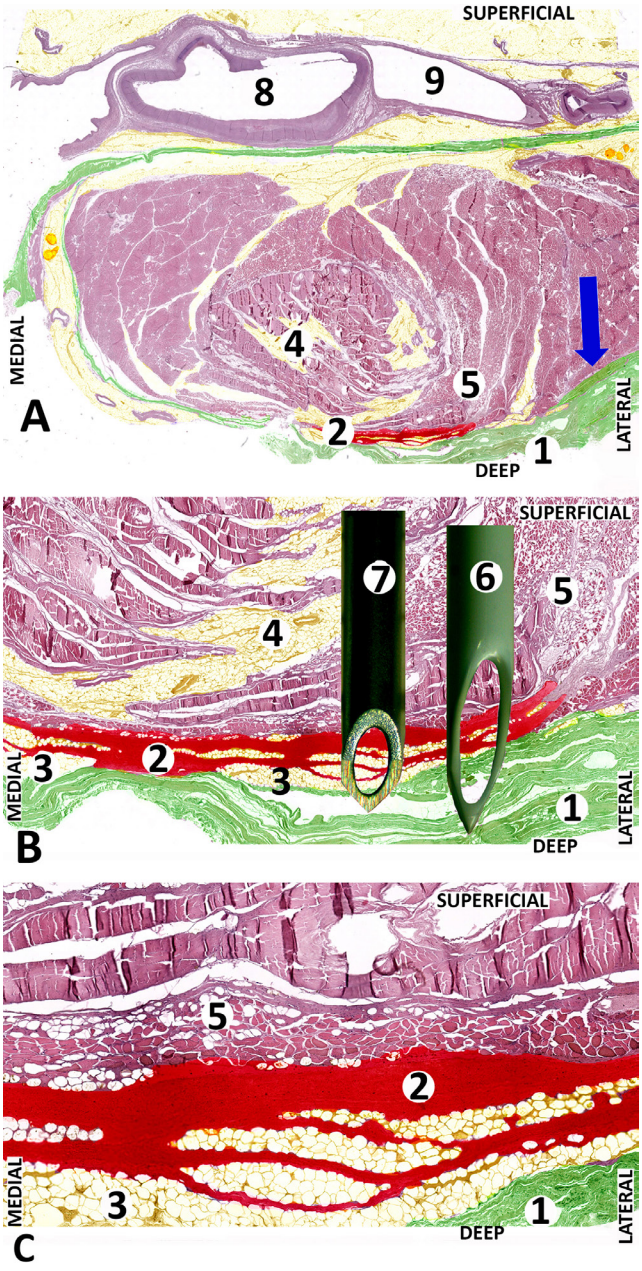

Figure 5 Histological distribution of injectate following a PENG block. Histological cross-sections were obtained just superior to the hip joint at the level of injection. (A–C) demonstrate PENG block injections using heparinized erythrocytes as a marker. (A) Global view showing distribution of the marker (red) within adipose tissue compartments. Some areas show muscle fascicles adherent to connective tissue (blue arrow), with no adipose tissue between them. (B) Higher-magnification detail from (A), where two needles were superimposed at the same magnification using Photoshop software. The needles are shown at an anatomical scale for comparison with the adipose tissue layer observed at the injection site. (C) Higher-magnification detail from panel A, demonstrating marker localization within adipose tissue between the epimysium and perimysium. 1, Capsule–periosteum complex (green); 2, Injected marker (heparinized erythrocytes); 3, Adipose tissue between capsule–periosteum complex and muscle; 4, Intramuscular adipose tissue; 5, Iliacus; 6, 22G 15° needle; 7, 22G 30° needle; 8, Femoral artery; 9, Femoral vein. Red staining indicates the distribution of the injectate (marker). Magnification: (A) ×20; (B) ×20; (C) ×60. Colors were digitally enhanced for clarity using Adobe Photoshop (Adobe, San Jose, California, USA). PENG, pericapsular nerve group.

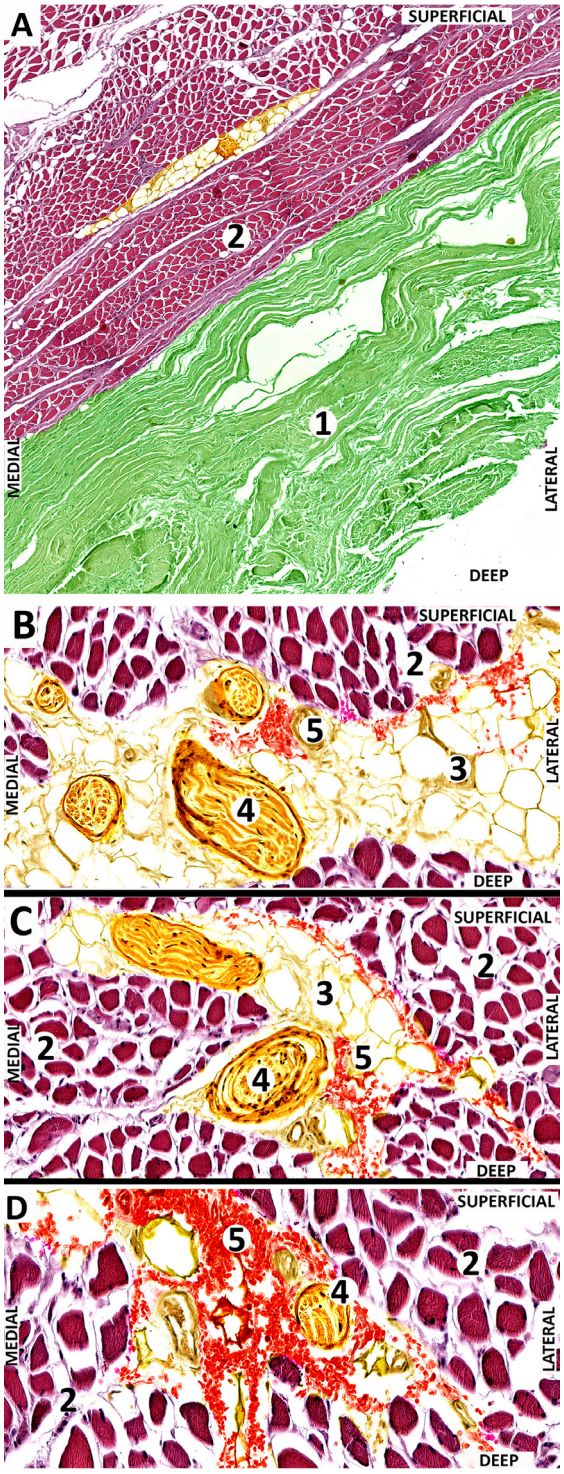

Figure 6 Intramuscular distribution of injectate following a PENG block (at the level indicated by the blue arrow in figure 5). (A) Capsule–periosteum complex–muscle interface, with minimal or absent intervening adipose tissue. (B–D) High-magnification views demonstrating injectate localization within intramuscular adipose tissue between muscle fascicles (within the perimysium). Small nerve branches and vessels are observed in close proximity to the injected marker. 1, Capsule–periosteum complex (green); 2, Iliacus; 3, Intramuscular adipose tissue (yellow); 4, Small intramuscular nerve branches (gold); 5, Injected marker (heparinized erythrocytes, red). Red staining indicates injectate distribution. Magnification: A, ×80; B–C, ×200; D, ×300. Colors were digitally enhanced for clarity using Adobe Photoshop (Adobe, San Jose, California, USA).

Table 1 Distribution of methylene blue on cryo-cross-sectional analysis after PENG injections

Specimen no./Hip side	IM/ASIS	IM/AIIS	IM Psoas major/pelvic brim	IM/ Lesser trochanter	Capsule
No. 1/Right	+++	++	++	–	+
No. 2/Right	+++	+++	++	–	–
No. 3/Left	+++	+++	++	–	–
No. 3/Right	+++	+++	++	++	–
No. 4/Left	+++	+++	+	–	–
No. 4/Right	+++	+++	+	–	–
No. 5/Left	+++	+++	++	–	–
No. 5/Right	+++	+++	++	–	–

Cryo-cross-sectional slides were obtained at the level of the ASIS, the AIIS, the pelvic brim adjacent to the iliopsoas tendon, the lesser trochanter, and hip capsule. Methylene blue spread was assessed using a 4-point scale: Important (+++), present (++), minimal (+), none (–).
AIIS, anterior inferior iliac spine; ASIS, anterior superior iliac spine; IM, intramuscular.

3. More distally, at the level of the acetabulum, methylene blue was observed within the iliopsoas muscle and in proximity to the femoral nerve region (figure 4C). At the level of the lesser trochanter, methylene blue was absent or minimal in most specimens.
4. At the level of the anterior hip capsule and femoral head-neck junction (figure 4D), methylene blue remained closely associated with the iliopsoas muscle, without apparent extension to the hip capsule or joint space.

Histological analysis

Histological evaluation was performed on four hips, two control specimens, and two marker-injected specimens (figures 5A–C and 6A–D).

Control specimens

Histological sections allowed clear identification of the iliacus, psoas major, and iliopsoas muscles, the femoral nerve, and the capsule-periosteum-complex, and the interface between the capsule-periosteum-complex and the iliac muscle. The enthesis, representing the attachment of the iliacus muscle to the iliopubic ramus, was well-defined and consisted of fibrous connective tissue continuous with the periosteum of the iliac bone. No clear histological boundary could be identified between the periosteum and the joint capsule, as both were composed of dense collagenous connective tissue. Therefore, these structures were considered a single capsule-periosteum complex.

Within the iliacus muscle, individual myocytes were invested by endomysium and organized into fascicles surrounded by perimysium. The entire muscle was enclosed by an outer connective tissue layer, the epimysium. Adipocytes, blood vessels, and nerve endings were identified between adjacent perimysial septa.

At the level of the enthesis, two histological configurations were observed. In some regions, adipocytes were present between the epimysium and the outermost muscle fascicles, adjacent to the capsule–periosteum complex. In other regions, adipocytes were absent, and the outermost muscle fascicles merged directly with the dense collagen fibers of the capsule–periosteum complex.

Marker-injected specimens

Heparinized erythrocytes were consistently identified within the epimysial compartment and along connective tissue planes between adjacent perimysial septa separating muscle fascicles. The marker was also present in adipose-containing spaces between these septa, together with small vessels and nerve branches. No marker was identified within the dense collagen fibers of the capsule–periosteum complex.

DISCUSSION

The combination of macroscopic dissection, cryo-cross-sections, and histological analysis provided complementary perspectives on injectate distribution following a PENG block and allowed a more comprehensive anatomical assessment than any single modality alone. While gross anatomical dissection demonstrated extension of methylene blue toward the femoral nerve and its branches, as well as apparent spread toward the anterior hip joint region, cryo-cross-sectional analysis consistently showed the dye remaining within the iliopsoas muscle compartment. Histological analysis further localized the marker to subepimysial and intramuscular adipose-connective tissue planes. Taken together, these findings suggest that injectate distribution following a PENG block occurs predominantly within the iliacus–iliopsoas muscle compartment and do not support preferential spread along a distinct musculo-osseous fascial plane.

The apparent discrepancy between periosteal staining on gross dissection and the predominantly intramuscular distribution observed on cryo-cross-sectional and histological analyses warrants careful interpretation. One possible explanation is secondary diffusion of methylene blue beyond the primary injectate compartment, resulting in superficial tissue staining without substantial injectate localization. Therefore, periosteal staining should be interpreted with caution and does not necessarily reflect substantial injectate distribution beyond the iliopsoas muscle compartment. The use of both methylene blue and heparinized erythrocytes provided complementary information regarding injectate distribution. While methylene blue may extend beyond the primary injectate compartment, erythrocytes remain localized and therefore more accurately reflect the site of bulk injectate deposition.

Moreover, the proximity of methylene blue to the femoral nerve observed on both gross dissection and cryo-cross-sections suggests that diffusion from the primary intramuscular injectate compartment may allow LA to reach the femoral nerve. The absence of a visible marker beyond the epimysial compartment does not exclude diffusion of LA molecules, as dye-based and contrast-based techniques primarily reflect bulk injectate distribution. Consequently, LAs may reach adjacent neural structures, despite the lack

of demonstrable pericapsular spread. Such diffusion may contribute to both the analgesic effects of the PENG block and to the quadriceps weakness reported in previous clinical studies.[6–11]

Histological analysis demonstrated that the marker was predominantly confined to adipose-containing connective tissue planes and was not identified within dense collagenous structures such as the epimysium, or capsule–periosteum complex. At the osteomuscular interface targeted by the PENG block, adipose tissue was limited and discontinuous, whereas dense connective tissue predominated. These observations suggest that injectate preferentially follows low-resistance adipose pathways and may be less likely to traverse dense connective tissue barriers.[12–16] Consequently, the microanatomical architecture of this region may help explain why the injectate remained largely confined to the iliopsoas muscle compartment rather than spreading between the muscle and bone.

Histological analysis also demonstrated the presence of small nerve branches within these adipose-containing connective tissue planes, supporting intramuscular neural blockade as a potential contributor to the analgesic effects of the PENG block.

Although several clinical studies have demonstrated the analgesic effectiveness of the PENG block, its anatomical basis remains debated.[17 18] The technique was originally described as targeting a musculo-osseous plane to facilitate spread toward the anterior hip capsule.[1 19] However, our previous CT-based study demonstrated that the injectate primarily distributes within the iliopsoas muscle.[2] The present study confirms and extends these findings through complementary anatomical and histological analyses, providing a more detailed understanding of injectate localization and its potential mechanisms of action.

This study has several limitations. Cadaveric injectate distribution may differ from in vivo conditions because tissue perfusion, muscle tone, and physiological pressure gradients are absent. Although histological analysis provided detailed information on injectate localization, distinguishing between adjacent connective tissue structures may be challenging in some regions. Furthermore, marker localization reflects bulk injectate distribution and does not necessarily replicate the diffusion characteristics of LA molecules. Only a single injectate volume (20 mL) was evaluated; therefore, the extent to which injectate distribution and potential femoral nerve involvement may vary with different volumes remains uncertain. In addition, the study did not specifically identify or trace individual articular branches supplying the anterior hip capsule, including branches from the femoral, obturator, and accessory obturator nerves. Consequently, the relationship between injectate distribution and these specific neural targets remains uncertain and warrants further investigation. Despite the relatively small number of cadaveric specimens, anatomical variability in this region is limited,[20] and the consistency of findings across three complementary methodologies—dissection, cryo cross-sections, and histology—supports the robustness of the observations.

CONCLUSIONS

Across all study modalities, the injectate following a PENG block was predominantly confined to the iliacus and psoas muscle compartments, without consistent involvement of the hip capsule. The findings support intramuscular neural blockade as a potential mechanism of analgesia and suggest that diffusion of LA from the primary injection site may contribute to femoral nerve involvement. Future studies should evaluate the relationship between injectate volume, anatomical distribution, analgesic efficacy, and unintended motor blockade in the PENG block.

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Contributors PG, XS-B, and MAR: These authors designed and conducted the study, analyzed results, wrote and reviewed the manuscript. AH, AP, NG, CV, ALB, and MH analyzed results, wrote and reviewed the manuscript. PG is acting as the guarantor.

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Competing interests None declared.

Patient consent for publication Not applicable.

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