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ORIGINAL PAPER

Topography and evidence of a separate "fascia plate" for the femoral nerve inside the iliopsoas – A dorsal approach

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Abstract

The femoral nerve stretch test is an essential part of clinical neurological examinations. This test is performed alongside Magnetic Resonance Imaging (MRI) to determine if there is any evidence of nerve root irritation, usually as a consequence of disc prolapse. The test occasionally gives false positive results. Why such false positives can occur, is subject to continued research, however, no obvious reason has yet emerged. We hypothesize that connectives of the femoral nerve may explain such a phenomenon. To see these connectives, we approached the femoral nerve from dorsal in 12 cases. With the use of ink injection into the subparaneural compartment of the femoral nerve and dissections, a thin transparent structure can clearly be seen that is separate from the epineurium, perineurium, and a paraneural sheath. A continuation of the paraneural sheath produces a fascia plate approximately 1.5 cm in width and with a thickness of around 3 mm, which not only circumnavigates the nerve but projects into the surrounding tissues. Our qualitative observations show that not only does this femoral nerve fascia plate exists, but it also contains nerves and vessels. Furthermore, we show that the femoral nerve is connected to the myofascial complex of the iliopsoas, and in a separate fascia plate from the iliopsoas fascia. This plate is a hitherto neglected connective which extends as far as the spinal dura mater. Evidence from our plastinates and histological sections suggests that when tension is applied to the femoral nerve during the femoral nerve stretch test, tension is also applied to the femoral nerve fascia plate. The femoral nerve fascia plate could be a specific factor that contributes to pain resulting in a false positive femoral nerve stretch test.

KEYWORDS
femoral nerve, iliopsoas muscle, Methylene blue, nerve sheath, paraneural sheath, psoas fascia

1 | INTRODUCTION

In a clinical environment, the femoral nerve stretch test is used in order to stress the femoral nerve (FN) and the mid lumbar (L2-L4) nerve roots. This helps medical practitioners diagnose nerve root impingements and upper lumbar disc herniation. This test is also known by various other names such as the Mackiewicz-test (Estridge et al., 1982), and a reverse Lasègue

sign (straight leg raising test for sciatic nerve impingement; M Das & Nadi, 2020).

Performing the femoral nerve stretch test, the patient lies prone and the knee is flexed. A positive test is when the patient suffers anterior thigh pain. However, false positives are known to occur when a patient also suffers from hip dysfunction, diabetic neuropathy and injury to the anterior thigh or iliopsoas complex.

To explain the mechanics of the femoral nerve stretch test and in order to explain why or when false positive results can occur, the nerve pathway must be clearly understood before a suggestion for the cause of pain is offered. This study intends to examine the anatomy surrounding the femoral nerve and shows that the presence of a large fascia plate, hitherto undescribed, could be a factor.

Many papers have been written about the structure of the peripheral nerves' fascial makeup (Krstic, 1988; Lang, 1961; Stecco et al., 2020; Van Beek & Kleinert, 1977; Vloka et al., 1996). Very few research papers specify the femoral nerve topography (Nobel et al., 1980) and a new perspective of the femoral nerve is achieved when dissecting dorsally. By way of qualitative anatomical dissections and ink injections we tried to figure out the connections of the femoral nerve to the myofascial complex of the illiopsoas or to the iliopsoas fascia, both of which are not completely visible from ventral.

2 | METHODS

2.1 | Removal of iliopsoas compartment from ventral

The femoral nerve (FN) was examined from nine fresh and three Thiel fixed cadavers (Hammer et al., 2015), aged between 80 and 99 years, 88.6 median years, nine females, three males (Table 1).

The following procedure was employed to cases 1-10, see Table 1.

The psoas major was located and carefully removed from its origins from the L2-L4 vertebrae to its insertion at the lesser trochanter, removing with it iliacus. Removal of iliacus requires scraping off the inside of the iliac fossa and cutting the origin of psoas major as close to the vertebrae as possible. The external iliac vessels were kept for orientation. After dissecting, the specimens were immediately labeled on the anterior surface at the inguinal ligament level.

2.2 | Ink injection and dorsal dissection

After removal of the specimens from the cadavers, the FN cases 1-10 were injected ventrally with Indian ink (Scribtol black –Indian-ink, Pelikan, Hannover, Germany; Table 1). This was injected into the subparaneural compartment of the FN with a 20 gauge needle, into a space described as being between the paraneural sheath and the epineurium (Karmakar et al., 2013). We injected each specimen at three different points—cranioventrally, at the level proximal to the division of the obturator nerve, and one inch above and below the inguinal ligament. If the FN was not obvious, then dissection anteriorly was undertaken in order to locate and access the FN. The amount of ink injected was between a minimum of 1.5 ml and a maximum of 5 ml depending on the tissues resistance when injecting the ink. After anterior ink injection, the iliopsoas compartment was flipped to approach from the dorsal aspect. Dissection of iliacus and psoas major was carefully undertaken to observe the FN pathway and its associated structures.

In two cases, we removed the FN with its plate for further quantitative data and one was used for further plastination (cases 19 and 20, see Table 1).

In case 9 the specimen was injected with blue resin (Technovit 7143, Haraeus Kulzer GmbH, Wehrheim, Germany) and dissected anteriorly (Table 1).

TABLE 1 Body donors. 12 dorsal dissections (including 2 in situ), 2 additional dissections for data collection, 3 used for ink experiment, 1 histology, 2 plastinations, total – 20

Body donor	Age	Gender	Notes	Case number
45/18	99	F	Thiel	1
75/18	88	F	Thiel	2
114/18	80	F	Fresh	3
2/19	88	M	Thiel	4
11/19	84	M	Fresh	5
12/19	98	F	Fresh	6
32/19	80	F	Fresh	7
38/19	91	F	Fresh	8
46/19	88	F	Fresh	9 - resin
101/19	93	F	Fresh	10
79/18	84	F	Thiel	11 - in situ
6/19	91	M	Thiel	12 - in situ
5/20	91	M	Fresh	13 - Histology
13/19	89	M	Fresh- pars psoatica	14 - ink exp
122/16	80	M	Fresh- Tractus	15 - ink expy
115/16	92	M	Fresh--Tractus	16 - ink exp
31/13	89	F	Anatomy collection	17 - Plastination
06/15	89	M	Anatomy collection	18 - Plastination
88/20	88	F	Fresh additional dissection	19 - Measurements/ Plastination
91/20	99	F	Fresh additional dissection	20 - Measurements

2.3 | Dissection in situ

In two cases with the cadavers lying prone (cases 11 and 12) we removed all spinal muscles including quadratus lumborum from dorsal. Further removal of the bony structures of the iliac fossa and the

acetabulum revealed the dorsal aspect of iliacus in situ together with the connectives (Figure 2). In case 11, the final specimen dissected was removed, dehydrated, and impregnated with polyethylene glycol 1000 (Merck, Schuchardt OHG, 85662 Hohenbrumm, Germany - see Video S1).

2.4 | Histology/Submacroscopy

Samples along the FN were taken from inside iliacus of case 13 with the adherent fascia layers (Table 1). These samples were fixed in buffered formaldehyde, paraffin-embedded, and stained with hematoxylin/eosin or Masson Trichrome dye as done by Reinas group (Reina et al., 2020). We also performed immunostaining against Von Willebrand factor (Anti-Human vWF, Goat, CoaChrom Diagnostics, Maria Enzersdorf, Austria), against Substance P (AB1566, Rabbit, EMD Millipore, Billerica, Massachusetts, USA), neurofilament (Neurofilament MAB5262, Millipore, Merck, Darmstadt, Germany), Collagen type 1 (Abcam ab 34710) and type 3 (Abcam 7778) and Epithelial Membrane Antigen (EMA), (Dako, E29, Agilent, USA; Reina et al., 2020).

To provide more quantitative data, measurements of the FN and its plate were undertaken from all samples including the plastinates (cases 17 and 18), the histology slices (case 13), all photographs and the dissection from two additional unfixed cases (cases 18 and 19, see Table 1). To accurately measure these specimens, we used a calliper (TCM Tchibo GmbH, Hamburg) and millimeter bars on the photographs.

We used two FN from our anatomical collection to perform a peripheral nerve teased-fibre test in which the connectives are macerated and the nerve fibers were stained (Krinke et al., 2000).

Plastinates were used to reveal the layout of the FN fascia plate. We used a standard plastination method and staining with periodic acid-Schiff (PAS) reaction as further described (Steinke et al., 2018). For additional quantitative insight we plastinated the dissection of case 19 (Steinke, 2001).

We scanned the slices using a high resolution scanner (600-1240 dpi, Scanner EPSON Perfection V750PRO, Epson Deutschland GmbH, 40670 Meerbusch, Deutschland).

2.5 | Ink experiment

When injecting Tinte ink into the FN, the ink travelled very quickly along the FN's pathway and into the surrounding tissues. This was undesirable. We wanted to find an ink that could show the existence of fascial borders and enclosed spaces surrounding the FN, so we designed an experiment to observe a dye that was unable to pass through a fascia membrane.

We used defrosted iliotibial tract specimens from cases 15 and 16, and one specimen of psoas fascia from cases 14 as a control (Table 1). We chose four different dyes: 1% Methylene blue (ThermoFischer, Kandel, Germany), Tinte 4001, Acrylic Ink, and Scribtol black –Indian- ink (Pelikan, Hannover, Germany).

Samples from the iliotibial tract were placed on top of a cotton pad inside a Petri dish. Two drops (40 µl) of each dye were placed on top of the iliotibial tract with the use of a micropipette. After 5 minutes the fascia was lifted off the cotton pad to see if any color had passed through the specimen. This process was observed three times with each dye.

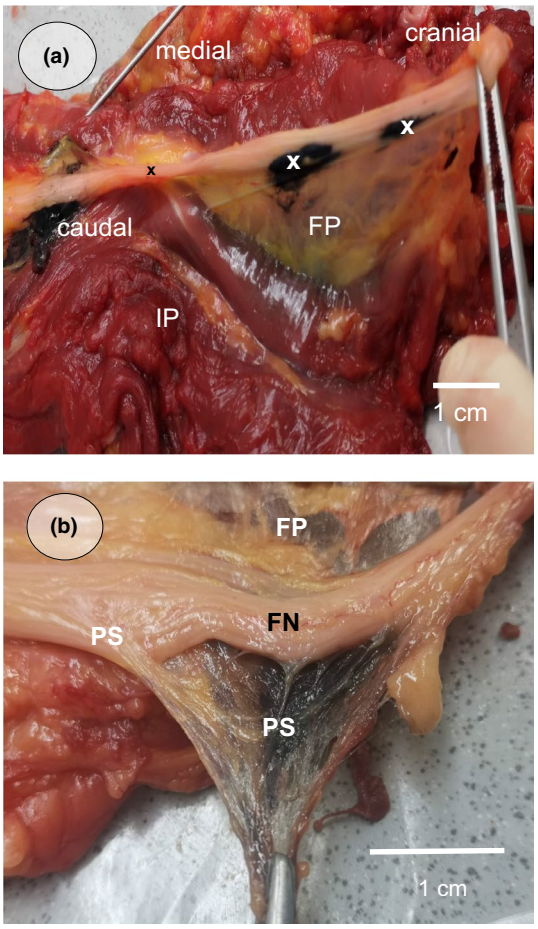

FIGURE 1 Dorsally dissected, fresh specimen (case 7, 80 years old, ♀) of a right side femoral nerve (FN) with the removal of the dorsal iliopsoas (IP). The distal part of the femoral nerve fascia plate (FP) is cut, to see the paraneural sheath. (a) Previous anterior ink injections (X) contained within boundaries along the nerve. Caudomedially the FP is seen entering into the iliopsoas which is removed from the fascia plate. The nerve is covered by remaining layers. The nerve, however, is seen not parallel aligned like a band, but slightly rotated (x seen to the left of the rotation). (b) In this higher resolution of the further dissection, the paraneural sheath (PS) can be distinguished covering the FN by its removal. The FP is, however, clean of Indian ink. Also the nerve adherent epineurium is clean. However, the outer layers contain the ink. This ink could be seen between the PS and other paraneural layers

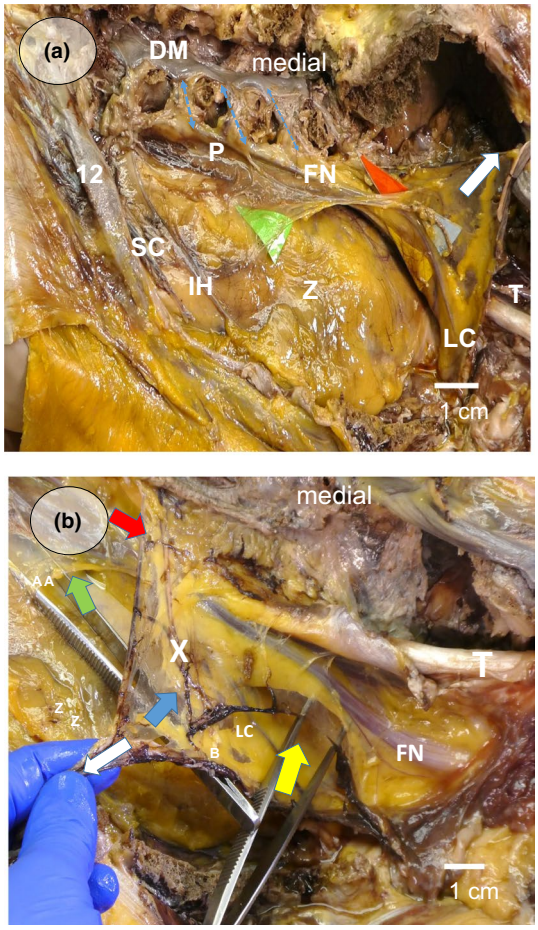

FIGURE 2 Thiel specimen, dorsal view, dissection in situ, left side (case 11, 84 years old, ♀). In the pictures cranial is left. The quadratus lumborum has been removed to see the dorsal coverages of psoas (P). Also the remnants of the iliac crest are removed. A white arrow represents the lifting of the former fascial attachments to the iliac crest. For orientation T represents the position of the psoas tendon. (a) Cranially the 12th rib is labeled (12). Caudal to 12, the subcostal nerve passes (SC). However, SC is covered by a white gleaming fascial layer. Within another layer sits the iliohypogastric nerve (IH). Then a layer reaches to cover the femoral nerve (FN) caudomedially on the dorsal surface of P. Green: A green paper triangle was inserted in-between the ventral portion of iliopsoas fascia and the retrorenal fascia (Zuckerkandl's fascia - Z). The green paper cannot be moved cranially or laterally, because of an attachment to Z. However, caudally this space is open. Red: The red triangular paper shows that the FN invaginates into a funnel, with the lateral cutaneous nerve of the thigh (LC). Blue: A blue triangular paper attached from cranial between the posterior and anterior surfaces of the iliac fascia, where previously fat has been set. The blue triangle cannot be moved medially as the LC is the medial border. To see this, the fascia was lifted dorsally (white arrow). Yellow: The yellow triangle is tricky to see (see 2b) and positioned anteriorly to the FN fascia plate and the posterior surface of the iliac fascia. This is because the blue triangle is anterior to the yellow with a fascial plate in-between. The lateral border of these adjoining fascias is the LC. Blue dotted arrows represent the connection between the spinal dura mater (DM) and our FN fascia plate via the dorsal aspect of the iliopsoas fascia; fascia psoas contain the FN. (b) We now see the posterior aspect of the view in 2a. Now the spaces created by the blue triangle in Figure 2a (now blue arrow) and yellow triangle (now yellow arrow) can be seen more clearly. By lifting the remnants of the iliac crest (white arrow) fascial coverages are placed back into their original place. The forceps have been placed in the space which is ventral to the FN fascia plate (yellow arrow). Ventral to this space the lateral cutaneous nerve of the thigh (LC) can be seen embedded in fat separated from the FN fascia plate. The red and green arrows denote the red and green triangles in the description in a. The deep circumflex iliac artery can be distinguished in a separate fold (Arterienscheide; white x)

3 | RESULTS

3.1 | Ink experiment

It was observed that the Tinte ink, the acrylic ink, and methylene blue had the ability to pass through the iliotibial tract. With the use of the control-case 14, this experiment also showed that methylene blue could pass through the much more delicate fascia of psoas fascia (data not shown). However, the Indian ink did not pass either the iliotibial tract nor the psoas fascia.

3.2 | Ink injections anteriorly and dissections dorsally

The injected Indian ink dye did not travel into the surrounding tissues of cases 1-8 and 11 but rather within a contained area around and alongside the FN (Figure 1a).

In dissections of the six fresh specimens, different fascia layers could be seen (specimens 3, 5, 6, 7, 8, and 9 see Table 1). The dye was held within a layer. This dye was not found inside the layer covering the nerve (epineurium), as on further dissection the nerve appears to be free of dye. The dye was found in another outer layer, surrounding the epineurium, alongside the FN (paraneural sheath; Figure 1b). No dye spread into the flat fascia layers arising from this sheath. Also no dye was seen soaking psoas major (PM) or iliacus (MI) nor into the surrounding tissues of the ilioinguinal region. Even with the maximum of 5 ml of Indian ink, the dye was prevented from soaking these surrounding tissues. In the cases 1, 2, and 4 the Thiel fixed specimens, Indian ink was observed tracking along the length of the nerve. In the six fresh cases the dye stayed within obvious pockets (Figure 1a). In case 9 (fresh) where the blue resin was used it also could be seen to track along the length of the FN (no figure).

3.3 | Dorsal dissections

3.3.1 | Cranial of the iliac crest

In the Thiel fixed specimens, cranially of the iliac crest, the FN was seen attached to the psoas fascia within the dorsal coverage of psoas (Figure 2a,b). However, it was not clear if the FN has a separate fascia sheet ventrally to the dorsal part of the psoas fascia. Cranially the

psoas fascia containing the FN attaches craniolaterally to another fascia containing the iliohypogastric nerve (Figure 2a).

This fascia containing iliohypogastric nerve in turn is attached to the diaphragmatic fascia via another fascia enveloping the subcostal nerve. To understand the 3D layout of these folds and spaces a specimen was made with polyethylene glycol (see Video S1).

In the dorsal view, the ventral part of the psoas fascia is seen attached laterally to the dorsal aspect of the retrorenal fascia (Zuckerkand's fascia). Between these two fascia surfaces a space is created, closed cranially, open caudally (Figure 2a). Medially the dorsal part of the psoas fascia attaches to the vertebral bodies, pedicles, and discs, all which can be seen after the removal of the vertebral arches from dorsal. At this point, these soft components can be seen to be attached to the spinal dura mater. In one in situ Thiel fixed case (case 10, see Table 1), traction to the outer soft tissues, that is, all those tissues sitting lateral to the vertebrae, resulted in a movement of the spinal dura mater.

3.3.2 | Caudal of iliac crest

In the 12 cases that we removed and the 2 cases in situ we saw the FN descending caudally and remaining enveloped by the dorsal

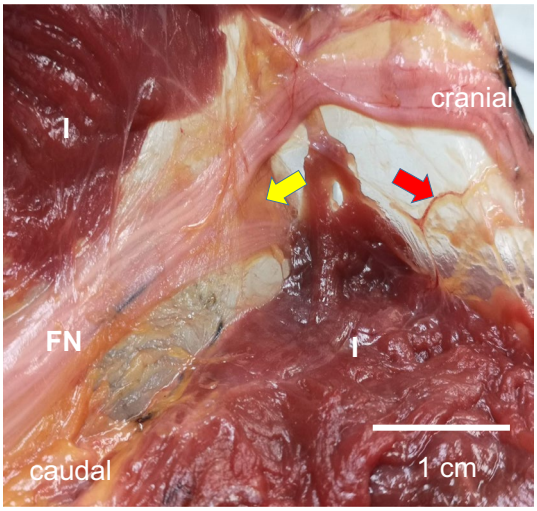

FIGURE 3 Dorsal approach, fresh specimen (case 7, 80 years old, ♀). After the removal of parts of iliacus (I) the femoral nerve (FN) can be seen sitting within its fascia plate. This plate was inside the muscle, covers the FN, and reaches again to the muscle. In this plate other structures can be distinguished, some of them bloody red (red arrow). The FN is the merging of two ventral rami inside this plate (yellow arrow). Medially it can be seen that fascia plate enters the muscle together with the FN

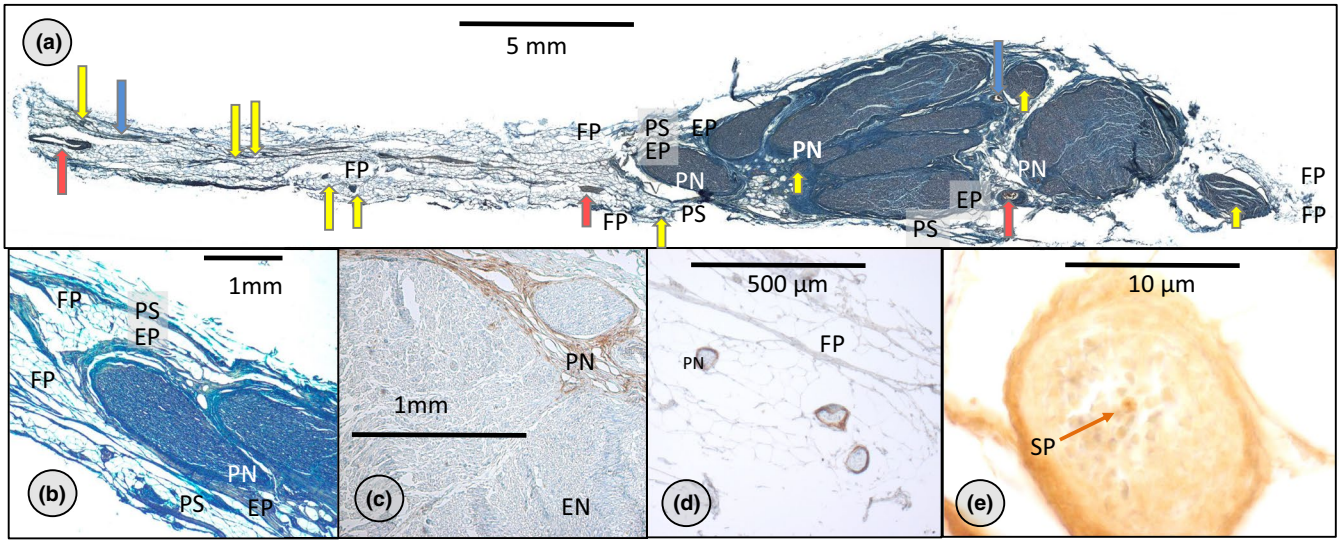

FIGURE 4 A histological view of a dissected femoral nerve with its fascial plate (case 13; 91 years old, ♂). (a) Scanned Masson trichrome slice, right femoral nerve ×10. Veins and arteries are seen contained within the brownish-blue stained nerve (blue and red arrows). The fascicles of the nerve (yellow arrows) are distinguished from the bluer connective collagen containing layers: the perineurium (PN) which is surrounding the fascicle, the epineurium (EP) which is surrounding the nerve itself, and another outer, looser layer, the paraneural sheath (PS) (ruptured on the upper side). Endoneurium is not optical dissolved in this magnification. From the outer PS another layer extends to the left and right. The collagenous connectives arising from the PS, we refer as to be the femoral nerve fascia plate (FP). These connectives contain nerves, arteries, and veins (yellow, red, and blue arrows). (b) In a higher resolution of another Masson trichriome (x20). Each fascicle is surrounded by the dark blue perineurium (PN). The nerve itself surrounds the epineurium, (EP). Another thinner, dark blue layer surrounds this all as a paraneural sheath, (PS). However, from the PS layers arises the femoral nerve fascia plate, (FP). (c) Epithelial Membrane Antigen staining (EMA) identified the perineureum cells of the N. femoralis (PN; brown). However, the endoneurium (EN) did not react resulting in empty spaces; ×50. (d) EMA staining of the FN fascia plate (FP) with nerves passing. While the smaller nerves in the plate shows perineurium (PN; brown). Neither the paraneural sheath nor the femoral nerve fascia sheet were positive for EMA; ×100. (e) Immunostaining against Substance P (SP) in a nerve inside the FN fascia plate. A small dot in the middle of this nerve could be a positive reaction for substance P; ×250

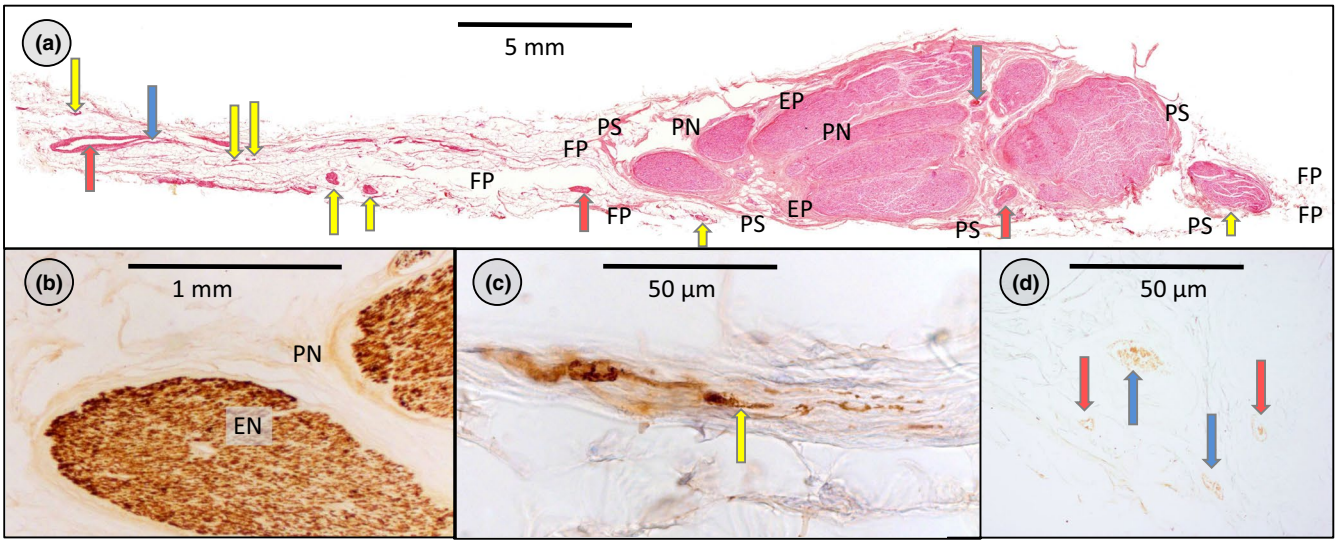

FIGURE 5 A histological view of a dissected femoral nerve with its fascial plate (case 13; 91 years old, ♂). (a) Scanned HE slice, right femoral nerve, 10×. Veins and arteries are seen contained within the pink stained nerve (blue and red arrows). The lighter orange stained coverages layers are seen: perineurium (PN), epineurium (EP), paraneural sheath (PS). Endoneurium is not optical dissolved in this magnification. From the outer PS another layer extends to the left and right. The structures arising from the PS we refer as to be the femoral nerve fascia plate (FP). It contains nerves, arteries, and veins (yellow, red, and blue arrows). (b) Neurofilament immunoreaction showing parts of the femoral nerve, 50×. In this resolution the perineurium (PN) appears as an unstained gap. Now, the gaps containing the unstained endoneurium are seen (EN). (c) Neurofilament immunoreaction showing nerves inside the FN fascia plate, yellow arrow, 150×. (d) Von Willeband factor immunostaining gives proof of vessels inside the FN fascia plate, blue, and red arrows for veins and arteries, 150×

aspect of the psoas fascia along with the lateral cutaneous nerve of thigh (Figure 2b). However, at the level of L4/5, iliac crest and the iliolumbar ligament, the dorsal aspect of the psoas fascia turns to cranial as it connects laterally to the iliac fascia (Figure 2a,b).

The ventral part of psoas fascia continues caudally by merging with iliac fascia creating the iliopsoas fascia. At which point a large amount of accumulative fat is observed between the two layers.

By the dividing of psoas fascia ventral and dorsal parts, a space is created that is open to caudal. This space is visible from dorsal and with the use of plastinates (Figures 2b and 6). This space contains psoas medially, the iliacus laterally, and FN in the middle. Ventrally, the iliopsoas fascia covers this space. However, in this space, the FN is seen enveloped by a fascia plate, which we refer to as the FN fascia plate (Figures 1-3 and 6). This fascia plate reaches lateral to the iliacus and medial to the psoas and is merged to these muscles. After removing these muscles, the FN fascia plate still exists and extends to these muscles (Figures 1a and 3).

Cranially this FN fascia plate attaches to the dorsal aspect of iliac fascia. This merging occurs near to the lateral cutaneous nerve of the thigh pathway which is encapsulated in the iliac fascia. Thus a lateral border is created for a space ventral of the FN fascia plate and dorsal of the dorsal aspect of iliac fascia (Figure 2). Such a separate FN fascia plate was seen in all 12 dissected cases (100%).

In the course of this dissection we have created a polyethylene glycol (PEG) impregnated 3D model which shows the FN fascia plate connections in situ (see Video S1).

Along the FN researched pathway the overall impression of the FN fascia plate's size is laterally not less than 1.5 cm in diameter (Figures 1, 3, 4, 5 and 6a) and it has a thickness of 3 mm nearest to the FN. These results are evident in all four of our quantitative data sources (plastinated samples, histology slices, photographs, and additional dissections).

In all cases we noted that the FN is rotated in its appearance. This phenomenon is revealed in the moment of opening iliacus and psoas from dorsal. A suggestion of this can be seen in Figure 1a. However, the peripheral nerve teased-fibre test was inconclusive with regard to inner fasciculation or rotation after maceration and staining of the fibers of FN (see Figure S1; Krinke et al., 2000).

When further dissecting the FN fascia plate, tracking along the dorsal aspect of the FN with a blunt end probe, starting from its origin, it can be seen that there are distinct layers surrounding the FN. When using a metal probe, these layers create a funnel like structure, a tube within a tube, like a telescope. Such spaces were also noticed after ink injections (see above). The connections of the FN fascia plate to the nerve were spider web in appearance, in between each layer.

3.4 | Histology/submacroscopy

A preliminary histological investigation supported our macroscopical findings and comparison to plastinates (Figures 4–6). The epineurium, the perineurium and the outer looser paraneural sheath surrounds the FN, and can be seen as distinct layers. One can observe that the FN fascia plate extends away from the nerve.

The plate contains nerves and vessels (Figures 4a,b, 5a and 6).

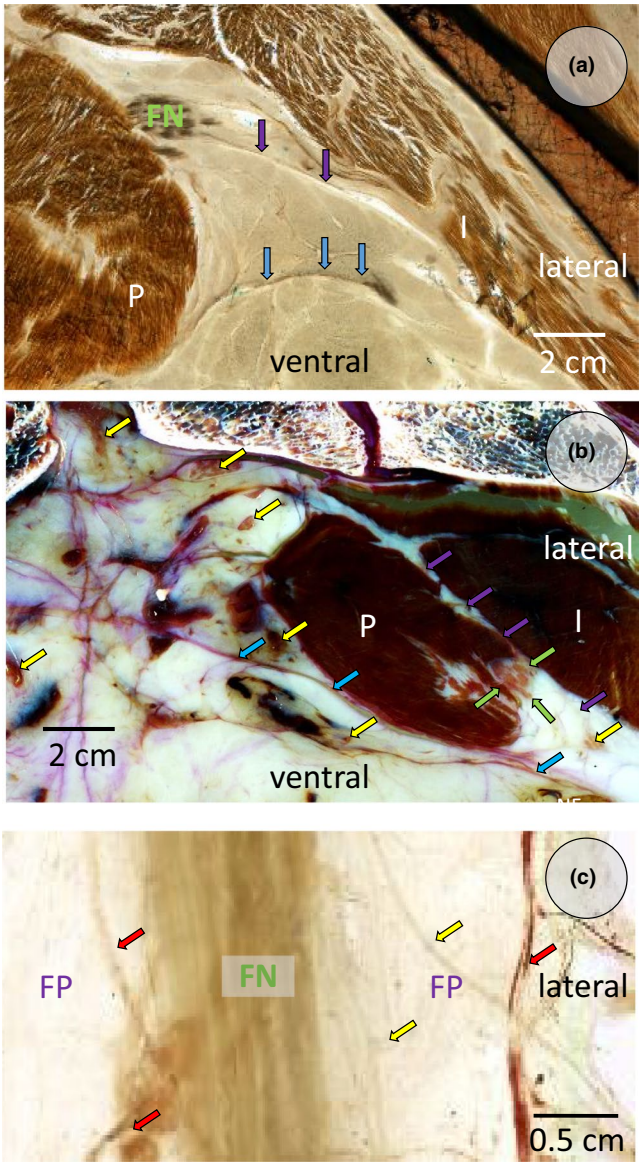

FIGURE 6 Plastination slices of femoral nerve (FN) with its fascial plate (purple arrows). Blue arrows indicate the iliopsoas fascia. (a) (case 17; 89 years old, ♀, E12 slice plastination) Purple arrows indicate the FN fascia plate extending from the FN. The FN can be seen sitting between psoas (P) and illiacus (I). (b) (case 18; 89 years old ♂, E12 slice plastination, collagen staining by PAS reaction) More cranial, the FN (green arrows) is to the left of the picture with the FN fascia plate sitting in-between I and P. The sacroiliac joint can be seen at the top of the picture. Yellow arrows indicate other nerves. Pink stained layers can be seen, showing fascia surrounding other nerves of the lumbar plexus (yellow arrows). (c) (case 19; 88 years old ♀, E12 flat embedded plastinated FN fascia plate) Centrally the FN reaches caudally. The connectives of the FN are transparent due to plastination. Thus the nerve fibers are visible. Plastination reveals comparable fibers (yellow arrows) in the FN fascia plate (FP). Another red type of lines reflects vessels (red arrows)

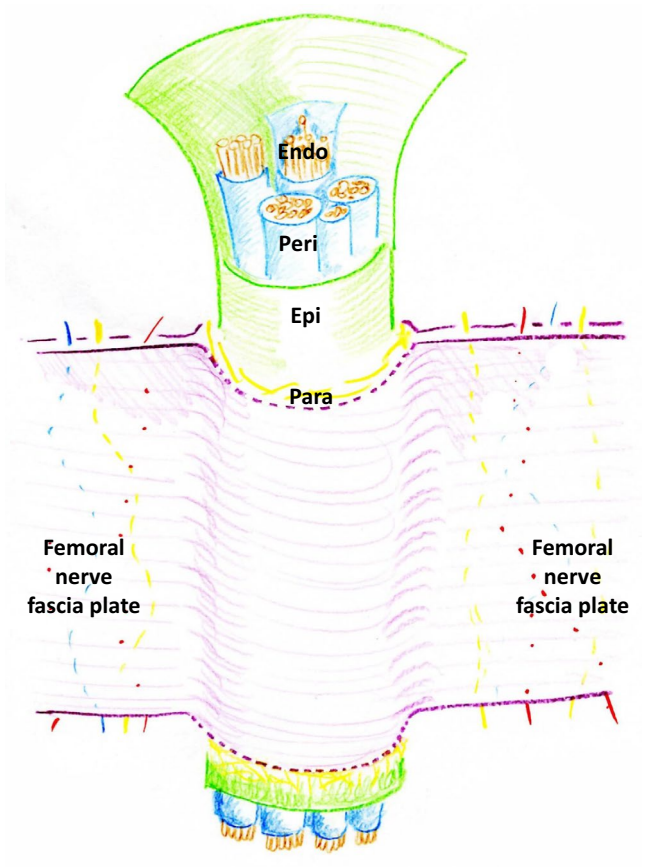

FIGURE 7 A schematic drawing of the femoral nerve, depicting the layering structures with regard to the femoral nerve fascia plate (purple). This plate contains nerves (yellow dots) and vessels (red and blue dots). The broken purple line is the most outer layer of the paraneural sheath which is attached to the plate. Endo - (brown) Endoneurium. Peri - (blue) Perineurium. Epi - (green) Epineurium. Para - (yellow) Paraneural sheath

The nerves inside the FN fascia plate showed positive reactions against neurofilaments along with the FN itself (Figure 5b,c). Inside the FN fascia plate, vessels were also detected using von Willebrandt factor (Figure 5d). Layers surrounding the FN were distinguishable, after using immunoreactions against epithelial membrane antigen (EMA; Figure 4c,d) and against collagen types (Figure S2). With the use of EMA we could identify perineurium cells specifically. The EMA reacted only with the perineurium cells surrounding the fascicles and nerves in the plate, while the EMA reactions were negative for the outer paraneural sheath and FN fascia plate itself (Figure 4d).

The Massons trichrome stain also shows that the FN and its surrounding layers contain collagen (Figure 4a,b). The FN fascia plate arises from the outer, looser layers of the collagenous connectives surrounding the nerve, the paraneural sheath. Proofs for collagen type 1 and 3 were unspecific. However, it could be seen that both types of collagen were evident in the FN fascia plate (Figure S2). There is also positive immunostaining against Substance P in a small nerve inside the FN fascia plate (Figure 4e).

Although nerves and vessels were qualitatively observed and located using histology and immunostainings further quantitative data of these structures were not collected.

Plastinates have shown the FN fascia plate apart from the iliopsoas fascia (Figure 6). In horizontal slices below the iliac crest the FN was seen embedded in such a plate. The plate was seen in-between the psoas, and the iliacus. This was the case cranially (Figure 6a) and caudally (Figure 6b). Submacroscopic stainings revealed collagenous layers surrounding other nerves of the lumbar plexus (Figure 6b). Nerves and vessels are also revealed by embedding the FN with its plate to E12 epoxy resin (Biodur, Heidelberg, Germany; Figure 6c).

Our macro- and microscopical findings are summarized in a schematic drawing (Figure 7).

4 | DISCUSSION

One major clinical feature of low back pain and radiculopathy is that it appears to be exceedingly common, and astonishingly in 85% of cases, no specific diagnosis can be made (Hartvigsen et al., 2018).

Despite modern technological diagnostic testing, the femoral nerve stretch test (FNST) remains an integral part of clinical differential diagnosis when screening for possible nerve root impingements. However, for a clinician to interpret these signs and symptoms, they require a good understanding of the femoral nerves (FN) static and dynamic anatomy.

By way of qualitative anatomical research, this study intends to identify what other factors or entities may be involved in creating pain, alongside or within the nerve. These factors will help strengthen previous theories and improve understanding regarding the specificity and sensitivity of the FNST.

4.1 | Nomenclature

The separate outer layers surrounding the nerves have been extensively described but while the nomenclature may differ (paraneurium, circumneurium, common epineural sheath, conjunctiva nervorum, adventitia, mesoneurium, and paraneural sheath), the descriptions remain similar (Krstic, 1988; Lang, 1961; Reina et al., 2020; Smith, 1966; Stecco et al., 2020; Van Beek & Kleinert, 1977; Vloka et al., 1996).

Steccos's group is mostly concerned with the study of fascia, and recently studied the "paraneural sheath" of the median nerve (Stecco et al., 2020). Therefore, and in keeping with our present research, and since we are focusing here on fascia, we will continue using the term "paraneural sheath" for the most outer layer of the nerves' gliding system.

4.2 | Layers and blood vessels

Our evidence shows that a paraneural sheath of the FN exists, but it has another external connection. This continues beyond just circumnavigating the nerve but extends into the periphery.

This extension is the FN fascia plate (Figures 2, 3, 5, and 6). We can see from our results that this plate is visible in all 12 dissected cases, 2 additional dissections, histology, and plastinates (see Table 1).

Such a continuation of a layer beyond the surrounding paraneural sheath is described by Smith et al. recognizing that peripheral nerves have a "mesoneurium" which is similar to the mesentery of the small intestine (Smith, 1966). In their work on the vasculature of the lumbosacral spinal nerve roots, Parke and Watanabe show a schema of a peripheral nerve (Parke & Watanabe, 1985). These papers both describe vessels coursing through an "epineural extension" or "mesoneurium," respectively. Their description of such an extension is similar to our finding of a FN fascia plate, as vessels are seen to exist in this plate as seen macroscopically and by positive reactions against von Willebrand factor (Figures 1a, 3 and 5d).

Reina, Boezaart, and others found that the paraneural sheath is interconnected with extensions from the adventitia of neighboring vessels (Reina et al., 2020).

These references to adventitia appear to be in keeping with Pirogov's older fascial sheath descriptions for blood vessels (Pirogov, 1860). Pirogov describes these "Arterienscheiden" as connecting the arteries to the periphery. The FN fascia described here plate passes medially to the deep circumflex iliac artery, which is seen passing inside another fascia (Figure 2b). Thus the FN fascia plate is apparently separate to "Arterienscheiden."

Kobayashi's group suggested that the FN's intraradicular blood can be influenced by tension placed on the nerve (Kobayashi et al., 2003). It is likely that the vessels seen merged to this FN fascia plate can be altered and possibly compressed in a FNST (Figures 4, 5a,d and 6c). We suggest that the FN fascia plate holding these blood vessels and nerves is likely to be a pain source.

4.3 | Comparable fascia plates

A comparable continuation of a layer beyond the surrounding paraneural sheath (a "mesoneurium") is also seen in a layout of the sciatic nerve inside the hamstrings shown by Andersen and colleagues (Andersen et al., 2012; their Figures 2c and 4c). Though they did not comment on this peripheral extension, it is shown in their figures, suggesting that even the sciatic nerve may have a comparable fascia plate.

Stecco's group, more recently, has shown a peripheral connection of the median nerve paraneural sheath (Stecco et al., 2020). In their Figure 4c, they name it as "an intermuscular septae, or epimysium," but only in the figures. The term intermuscular septae is normally applied to aponeurotic sheets separating various muscles, which may be the case in Stecco's group subject of research, and even in ours. However, our results clearly show the FN is held by a fascia plate connecting it to the surrounding muscle (Figures 1, 3, and 6). Moreover, the nerve and the plate is not only inside but also dorsally of the muscles. Thus such a FN fascia plate would be considered more a perimysium than an endomysium. Unfortunately,

these terms do not reflect the anatomy of such a FN fascia plate. In the region cranially to the iliac crest, we have seen the FN enveloped by the dorsal aspect of iliopsoas fascia along with the lateral cutaneous nerve of the thigh (Figures 2 and 6). Therefore, the term "endomysium" may also insufficiently describe the FN fascia plate's topography in this region below the iliac crest.

Our results clearly show that the FN's paraneural sheath has an extension: the FN fascia plate. This idea is summarized in our schematic Figure 7. Also, the polyethylene glycol (PEG) impregnated model gives a 3D model of such an extension (see Video S1): While a computed 3d model is error prone due to segmentation and reconstruction, the original specimen reflects the natural anatomy.

Finally, it was observed in the dorsal approach that other divisions of the lumbar plexus appear to be enveloped within their own "fascia plates." Figure 6b and the Video S1 shows the lateral cutaneous nerve of the thigh appearing to be located in its own fascia plate. Our results may support Thompson and Rorie's idea that "a fascial compartment is created for each nerve" (Thompson & Rorie, 1983, p. 120; Figure 3). Such compartments may be anatomical entities, as our findings suggest for FN. However, such compartments' fascial borders can also be followed by using horizontal plastinates (Figure 6).

4.4 | An external and internal gliding system

Descriptions of gliding systems of nerves often refer to connectives immediately surrounding a nerve (Clarke & Bearn, 1972; Lang, 1961; Stecco et al., 2020; Van Beek & Kleinert, 1977). These connectives refer to the nerves' ability to tolerate or restrict motion and stretching along its pathway. Evidence of such layers is found during dissections as telescopic tubes within tubes (no figures; Lang, 1961) as well as in our ink experiments, which show borders and spaces (Figure 1). These layers appear to be internal or separate to our fascia plate as no ink was seen in the FN fascia plate.

To differentiate these inner layers from the FN fascia plate, the volume and type of dye seem to be relevant, as we see in our experiments and other research papers (Nielsen et al., 2018). We noted that in previous research the dye quantity can be very high, up to 5 ml (Nielsen et al., 2018). Subsequent ballooning could break fascia layers and produce artefacts. The FN fascia plate was never ballooned (Figure 1a) and not in the FN itself or even when injecting ink near the FN (Figure 1b). Therefore, we think the FN fascia plate may not be a part of such gliding systems.

4.5 | Methylene blue vs Indian ink

Anderson's group and many others used methylene blue for their nerve research (Andersen et al., 2012; Thompson & Rorie, 1983). Our ink experiments, showed that methylene blue behaves in the same way as an anesthetic: It spreads into all surrounding tissues. For us the problem is that in doing so, it also penetrates thick fascia like the iliotibial tract (data not shown). For this reason, we suggest it is not a suitable ink for showing anatomical fascia borders or boundaries.

When we use Indian ink, a differentiation between the outer FN fascia plate from any paraneural sheath (mesoneurium) or from the epineurium could be established (Figure 1), as summarized by Figure 7.

4.6 | Layout of the FN fascia plate

We show, with the use of gross anatomical dissections, dye injections, plastinates, and histology, that there is a "fascia plate" connected to the outer layers of the FN, which provides connections and creates spaces (Figures 1–6).

Nerves are seen merged to the FN fascia plate in our preliminary histological survey and in plastinates (Figures 4–6). The FN fascia plate may consist of collagen type I or III to an unclear quantitative extent (Figures 4a,b, 5a and 6b, Figure S2). Vessels and nerves exist in this collagenous plate, as seen by positive reactions against Von Willebrand Factor and neurofilament (Figure 5b-d). Substance P could also be seen inside the FN fascia plate, a peptide neurotransmitter responsible for modulating pain sensitivity (Figure 4e). The use of epithelial membrane antigen (EMA) and neurofilament reactions has helped locate and identify specific layers in the small nerves merged within our FN fascia plate (Figures 4c,d and 5b,c). We can see the perineurium (with the use of EMA staining) clearly surrounding the FN but also surrounding these smaller nerves held within the FN fascia plate (Figures 4d and 6c).

More quantitative data could substantiate our suggestion that the nerves merged to the FN fascia plate could have synapses and thus could be responsible for pain, for example, when performing the FNST.

It is already discussed that the perineurium is a continuation of the dura mater (Haller & Low, 1971). These histological findings within the FN fascia plate may suggest indirect connections to the central nervous system. Figure 2a and the 3d model (see Video S1) show macroscopic connections after removing the bones and other ligaments. Such macroscopical dissections show the "connective tissues" of the FN, including the first qualitative description of the FN fascia plate.

4.7 | Dorsal approach

All previous research, both anatomical and clinically normally approaches the FN from ventral (Nielsen et al., 2017). Dorsal dissection shows the vectors of embryological development of the FN (from dorsal to ventral; Figure 3).

It shows the complex anatomical arrangement of the differing fascias especially in the area of L5 (see Video S1).

It may seem unobvious to look from dorsal; however, the lower limb rotates medially 90 degrees during development and, as a result,

the FN derives its roots from the posterior cords and the obturator nerve from the anterior cords in contrast to its final topography (Hamilton et al., 1978). The thigh muscles migrate from the lumbar area and carry their nerve innervation with them, hence L1-L4.

The ability to see the FN's rotation element macroscopically during dorsal dissection becomes much more apparent (Figure 1a). Whether this is from embryological origin or purely positional due to the surrounding musculature is debatable and requires further more research. The peripheral nerve teased fibre test has given us a minimal indication of fibre orientation, however, this is not the main topic of the present study (Figure S1; Krinke et al., 2000).

5 | CONCLUSION

In conclusion, the FN fascia plate is a thin transparent fascia seen to contain the FN with its divisions and smaller nerves (Figures 3, 4d,e, 5c and 6c).

Preliminary histological research suggests evidence for substance P (Figure 4e). Further research may show to what quantitative extent substance P exists in the FN fascia plate or in comparable layers which can be reached on the dorsal approach (Figures 2 and 3, Video S1) or by cutting plastinates (Figure 6).

During the FNST, the FN's internal and external layers may experience full tension, subsequently causing a minor distortion of this FN fascia plate. Creating a tension since gross movement is unlikely due to the parallel flat alignment of this plate along the axis of the paraneural sheath or mesoneurium. We could conclude that the FN fascia plate may also be a contributing factor in the cause of pain and therefore result in a false positive FNST (Figures 2b, 4a, 5a, 6 and 7).

We believe that when the connectives are altered, that is, any change in the collagen (Figure S2), this could lead to a more subtle tensioning of the FN fascia plate. These changes could occur after a trauma or in diseases like diabetic neuropathy, or even after hormonal changes (Goodman, 1954). The correlation of sex hormones to laxity is also recently described (Graf et al., 2019). Such connective changes to the FN fascia plate may also be related (cause and effect) to insufficient blood flow, which, as Kobayashi's group stated for the FNST, correlates to femoral pain (Kobayashi et al., 2003).

Our findings show that the FN fascia plate is an integral part of the FN. Therefore, any understanding of the FN should consider the FN connectives, not just the nerve itself. Clinicians should consider the anatomy of the FN fascia plate when searching for sources of pain.

6 | LIMITATIONS

The findings of our preliminary microscopical research is restricted to few histological examples. Just two in situ dissections looking above the iliac crest, from which we obtained an overview of the lumbar plexus fascia plates.

Also, the use of samples from the geriatric population for our specimens restricted the evidence that we were able to investigate in this study.

While this study was the first qualitative anatomical research showing a new entity connecting the FN to its surroundings, this lacks on quantitative data. However, this gives the outlook for further research.

We confirm that we have read the position of the Journal of Anatomy on issues involved in ethical publication and affirm that this report is consistent with the Journal's guidelines.

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CONFLICTS OF INTEREST

None of the authors has any conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Charlotte Kulow: contributed to the concept, the dissections and wrote the manuscript. Andreas Reske, Ingo Bechmann and Mario Leimert: drafting of the manuscript, giving clinical advice and critical revision, Hanno Steinke: contributed to concept, dissected and drafted the manuscript, Karsten Winter: reproduction/scanning of histology slides.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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