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Further defining subcutaneous innervation patterns to help reduce sensory deficits following ACL surgery using cadaveric heatmap and exploring ultrasound imaging

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Thesis

FURTHER DEFINING SUBCUTANEOUS INNERVATION PATTERNS TO HELP REDUCE SENSORY DEFICITS FOLLOWING ACL SURGERY USING CADAVERIC HEATMAP AND EXPLORING ULTRASOUND IMAGING

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ABSTRACT

Current ACL reconstruction techniques are performed with little regard to cutaneous nerves despite the evidence that iatrogenic nerve injury often leads to painful neuromas, reflex sympathetic dystrophy, or anterior knee/kneeling pain which directly impacts patients whose profession requires kneeling (Cerulli et al. 2013; Ruffilli et al. 2016; Hurley et al. 2018). In this study, the femoral cutaneous and saphenous nerves from two embalmed lower extremities were dissected in exquisite detail to create topographic maps of the anterior thigh and knee, detailing the nerves' anatomical course and relationship to relevant landmarks. To further characterize the cutaneous innervation at the knee joint, ultrasound techniques to locate sensory nerves were also explored. In both specimens, all branches of the femoral cutaneous and saphenous nerves were located, dissected, and mapped. In addition, the ability to locate terminal branches of the anterior femoral cutaneous nerve in a cadaveric specimen on ultrasound was successfully demonstrated. However, due to the small sample size, further research implementing similar methods is necessary to ensure the results are representative of the population and can be applied to surgical innervation. These findings highlight the importance of considering cutaneous nerves in ACL reconstruction techniques to minimize the risk of iatrogenic nerve injury and associated complications.

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LIST OF ABBREVIATIONS

ACL Reconstruction .... ACLr

Anterior Cruciate Ligament .... ACL

Anterior Femoral Cutaneous Nerve .... AFCN

Anteromedial bundle (of the ACL) .... AM

Bone-Patellar Tendon-Bone Graft .... BPTB

Central Nervous System .... CNS

Computerized Tomography .... CT

Dorsal Root Ganglion .... DRG

Hamstring Tendon Graft .... HT

Infrapatellar Branch of the Saphenous Nerve .... IPBSN

Lateral Femoral Cutaneous Nerve .... LFCN

Medial Femoral Cutaneous Nerve .... MFCN

Middle Genicular Artery .... MGA

Peripheral Nervous System .... PNS

Posterior Cruciate Ligament .... PCL

Posterolateral Bundle (of the ACL) .... PL

Quadriceps Tendon Graft .... QT

Sartorial branch of the Saphenous Nerve .... SBSN

Tibial Tuberosity .... TT

Ultrasound .... US

INTRODUCTION

Knee injuries such as fractures, dislocations, ligament/tendon damage, etc., are common across all age groups and populations. The major difference between younger and older populations, specifically, is the associated mechanism of injury in which knee damage is sustained. In younger individuals', injury is often seen during sporting events and exercise with older patients tending to have injuries related more to degenerative diseases, such as arthritis (Abhishek Vaish 2020). One of the most common sports/exercise-related knee injury involves complete or partial tearing of the anterior cruciate ligament (ACL). Following age and sex adjustment, the annual incidence of ACL tears shows 68.6 out of 100,000 individuals will suffer from an ACL injury (Abhishek Vaish 2020). Sports medicine physicians have estimated that biological females have an eight to nine times greater incidence of ACL ruptures compared to biological males (Prodromos et al. 2007).

There are several procedures and graft types that can be used but no graft type provides a performance/functional advantage (MOON Knee Group 2022). The ideal ACL graft choice is still up for debate and is usually based on surgeon preference. Following surgical reconstruction patients often report sensory deficits around their knee joint which is a result of iatrogenic damage to peripheral cutaneous nerves. Damage to these nerves from the skin incision or surgery can lead to variable sensory deficits such as hypoesthesia, dysaesthesia, or painful neuromas. These deficits have been linked to anterior knee/kneeling pain which could affect a patient's activities of daily living or impact patients with professions that require kneeling, such as builders, plumbers, mechanics, etc. (Cerulli et al. 2013; Ruffilli et al. 2016). Current surgical intervention to the knee joint is performed with little regard to the preservation of the cutaneous nerves despite the evidence of their impact on patients when damaged (Cerulli et al. 2013; Ruffilli et al. 2016; Hurley et al. 2018). With the emergence of minimally invasive techniques, the rate of damage to the cutaneous nerves during hamstring autografts was worse compared to open techniques (Sanders et al. 2007; Ruffilli et al. 2016).

The cutaneous innervation surrounding the knee joint is supplied by three terminal sensory nerves from the femoral nerve and lateral femoral cutaneous nerve (LFCN). The anterior femoral cutaneous nerve (AFCN), medial femoral cutaneous nerves (MFCN) and saphenous nerve from the femoral nerve combine with the LFCN to form a "patellar plexus". The AFCN and MFCN's combine to supply sensory innervation to the anteromedial thigh with the saphenous nerve and branches supplying the skin over the anteromedial aspect of the knee (Sabat and Kumar 2013; Kalthur et al. 2014). Currently, there is a lack of literature and research studies investigating the femoral cutaneous innervation pattern around the knee joint, with the majority of anatomical studies investigating the relation of these nerves to landmarks for hip surgery (Surucu et al. 1997; Ropars et al. 2009).

ACL ANATOMY, DEVELOPMENT, and FUNCTIONS

The first mention of the anterior and posterior cruciate ligament can be dated as far back as 3000 BC. Between 460-370 BC Hippocrates was the first to describe the laxity in the knee joint seen following cruciate ligament injury (Scheffler 2012). But it wouldn't be until the 1930's when the first English detailed description of the anterior cruciate ligament was presented by I. Palmer in his thesis (Scheffler 2012).

Embryologically the knee joint begins to develop during the 4th week of gestation with the formation of the cruciate ligaments developing from a concentration of synovial mesenchyme between their respective attachment sites as early as six to eight weeks (Scheffler 2012). During development, the mesenchyme is surrounded by synovium which eventually develops into the synovial membrane positioning the ACL outside the synovial cavity but within the joint capsule. The fully developed ACL is attached medially to the anterior intercondylar eminence of the tibia and ascends posterolaterally through the knee joint, twisting on itself externally, before expanding to attach on to the posteromedial aspect of the lateral femoral condyle (Markatos et al. 2012). The ACL receives its vascular supply from a branch of the middle genicular artery (MGA) a branch from the popliteal artery. The MGA enters the posterior/dorsal joint capsule dividing into several intra-articular branches supplying the soft tissue structures of intercondylar fossa (Scheffler 2012). A large posterior descending branch of the MGA supplies the ACL at the junction of the joint capsule, distal to the infrapatellar fat with the vascular supply of the ACL as a whole, decreases from proximal to distal (Scheffler 2012). The majority of the innervation of the ACL is through the posterior articular nerve which is a branch of the tibial nerve (Abhishek Vaish, 2020).

The ACL along with other structures of the knee joint support it during arthrokinematics and osteokinematics movement. These in combination allow the knee joint to have six degrees of freedom: flexion/extension, abduction/adduction, internal and external rotation and three translational motions, anteroposterior, mediolateral, proximodistal (Scheffler 2012). The primary function of the ACL is to restrict anterior tibial translation/posterior femoral translation during full range of motion and secondarily to resist internal/medial rotation. Histologically, the ACL is composed of longitudinally oriented collagen fibers (90% type I, 10% type III) and is formed by two distinct functional and anatomic distinct bundles: the anteromedial (AM) and posterolateral (PL) bundle (Scheffler 2012; Markatos et al. 2012). The AM bundle provides anteroposterior stability and is tight in flexion with the PL bundle providing rotational stability and is tight in extension (Scheffler 2012). Typically, the ACL is torn or injured during sudden twisting of the knee while the foot is planted or more commonly during sudden deceleration which leads to hyperextension of the joint (Abhishek Vaish 2020). Clinically an ACL tear can be diagnosed using imaging CT/MR or using clinical tests such as the Lachman, anterior drawer, or pivot shift test (Abhishek Vaish 2020).

ACL RECONSTRUCTIONS TECHNIQUES

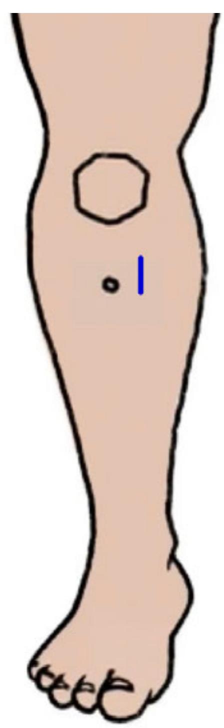
Following positive diagnosis of an ACL tear, a patient has several options including conservative non-surgical treatments or surgical repair. The non-surgical approach involves aggressive rehabilitation, typically in the form of physical therapy, and pain management (Abhishek Vaish 2020). With active patients' surgical intervention is required to regain full function of the ACL and restore knee stability. There are several procedures and graft types that can be used but no graft type provides a performance advantage (MOON Knee Group 2022). The ideal ACL graft choice is still up for debate and is usually based on the surgeon's preference. Many other aspects of ACL reconstruction (ACLr) depend upon the surgeon's preference including tunnel placement, use of a double or single bundle technique, and the type of fixation (Bonasia and Amendola 2012). But the overall goal of ACLr remains the same across all surgical options. The reconstruction ACL should be able to reproduce the histological and biomechanical characteristics of the native ACL, while also trying to incorporate the new ligament fully and quickly within the bone tunnels, have little to no risk of rejection or disease transmission, minimal donor-site morbidity, correct graft length and diameter, be cost-effective and readily available to all demographics and economic classes (Bonasia and Amendola 2012). Overall, there are three categories of grafts for ACLr autografts, allografts, and synthetic grafts.

Allografts are obtained from several places in cadaveric specimens including the Achilles' tendon, patellar tendon, hamstrings, quadriceps tendon, fascia lata, or the tibialis anterior/posterior (Bonasia and Amendola 2012). Allografts in the athletic and active population are rarely used due to the graft having a three times greater risk of retearing compared to autografts (MOON Knee Group 2022). But allografts do have some advantages over other ACL reconstruction techniques including a faster immediate post-operative recovery, less postoperative pain, a wide variety of graft sizes and shapes, and no need for graft harvesting which can lead to no donor-site morbidity and improved cosmetics (Bonasia and Amendola 2012). Historically the hamstring tendon graft (HT) and the bone-patellar tendon-bone graft (BPTB) have been the standard autograft with the quadriceps tendon graft (QT) gaining more traction in the sports medicine field. Autografts are obtained from the patient and offer several benefits over allografts and synthetic grafts. Some advantages of an autograft include improved stability, lower graft failure rate, lower infection rates, no risk of infectious transmission, and no risk of immune reaction (Bonasia and Amendola 2012). Synthetic grafts are classified as either scaffolds, which are made of synthetic tissues that stimulate fibrous growth, stents, which are designed to protect the healing of the graft during its incorporation into the bone, or prostheses, a polyethylene substitute to a biologic graft (Bonasia and Amendola 2012). The complication rate with synthetic grafts compared to allograft and autograft techniques is much higher. The scaffolds have been associated with synovitis, lack of fibrous growth, and failure to adhere to the bone tunnel (Bonasia and Amendola 2012). The prosthetic and stent-grafts also have a higher complication rate and have shown increased chronic knee instability, joint effusions, and synovitis (Bonasia and Amendola 2012).

Hamstring Tendon Graft

Autogenous hamstring grafts are a popular graft source for ACLr as it meets patient expectations, and the technique has become more minimally invasive (Sabat and Kumar 2013). During the procedure, the patient is placed in a supine position with the knee self-supported in 80-90° of flexion (Longino and Giffin 2012). After the area has been trimmed of hair the locations for a standard superior-medial outflow portal and anteromedial and anterolateral para-patellar ports are marked (Longino and Giffin 2012). Next, the main incision site for harvesting and drilling is marked and confirmed by palpating the pes tendons before the incision (Duplantier and McCulloch 2016a). In general, the incision is approximately two to three cm in length measured three finger breaths distal to the joint line, one thumb or two finger breaths medial and slightly distal to the TT **(illustration 1)** (Longino and Giffin 2012; Duplantier and McCulloch 2016a). The first skin incision is made at the hamstring graft portal, the skin edges are reflected, and subcutaneous structures are dissected (Longino and Giffin 2012). Next, the sartorial fascia is identified and the gracilis and semitendinosus tendons can usually be palpated just deep to the fascia (Longino and Giffin 2012; Frank et al. 2017). At this level, the gracilis tendon is located superiorly relative to the semitendinosus

Illustration 1: HT graft main skin incision

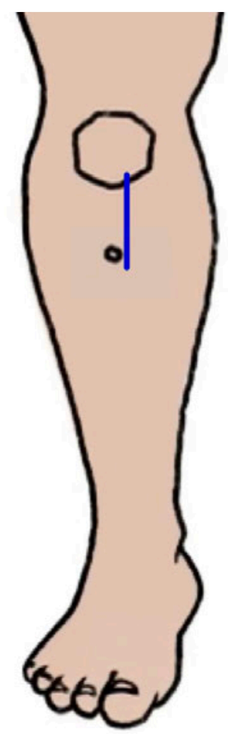


tendon and the semitendinosus tendon is usually larger in diameter (Longino and Giffin 2012). Next, an incision is made into the sartorial fascia in line with the tendons. The edges of the cut sartorial fascia are reflected with the tendons adhered to the underside of the fascia (Longino and Giffin 2012; Frank et al. 2017). Using tissue forceps and Metzenbaum scissors, the gracilis and semitendinosus tendons are dissected free from the sartorial fascia (Longino and Giffin 2012; Frank et al. 2017). Once the distal insertion of the tendons has been freed from the fascia, care is taken to ensure at least 10 cm of the tendon is free from an adhesion, particularly the semitendinosus tendon can have large tissue bands attached to the medial head of the gastrocnemius, ensuring the tendons do not prematurely amputate while using the tendon stripper (Frank et al. 2017). Also, at this time care should be taken to not blindly run instruments up the superior aspects of the tendons which may risk damage to the saphenous nerve (Longino and Giffin 2012). Next, an open or closed-ended tendon stripper is passed in line with the orientation of the tendon to release each from its proximal musculotendinous junction (Longino and Giffin 2012; Frank et al. 2017). Once a tendon has been stripped from the muscle belly, the tendon stripper must be carefully removed along the same orientation to avoid damage to neighboring neurovasculature structures (Longino and Giffin 2012). Lastly, once both tendons have been freed from their respective muscle belly the common insertion of both the tendons, pes anserine of the tibia, is freed, and the graft is placed in saline solution for preparation (Longino and Giffin 2012; Frank et al. 2017).

Bone-Patellar Tendon-Bone Graft

The BPTB is similar to the HT graft in that it is an autograft performed under general anesthesia. The same preoperative steps take base including, including trimming the knee of hair and positioning the patient supine but with the knee straight instead of flexed (McAllister and Wu 2012). Once under general anesthesia, the Lachman's, anterior drawer, and pivot shift test are performed to confirm an ACL tear (McAllister and Wu 2012). Following the confirmation, the incision sites for graft harvesting are marked. The first incision is a 5-7 cm longitudinal/vertical incision centered and marked over the patellar tendon extending from one cm medial to the inferior/distal pole of the patella down to one cm medial of the TT **(illustration 2)** (McAllister and Wu 2012; Duplantier and McCulloch 2016b). Similar to the HT graft, arthroscopic ports are marked but the BPTB graft uses a different port location. This procedure uses a superolateral, anteromedial, and anterolateral, with the anteromedial port coming from the main incision site (illustration 2) (McAllister and Wu 2012). Sharp dissection is used at the main incision site to cut the skin and subcutaneous adipose tissue down to the level of patellar tendon paratenon (McAllister and Wu 2012).

Illustration 2: BPTB graft main skin incision

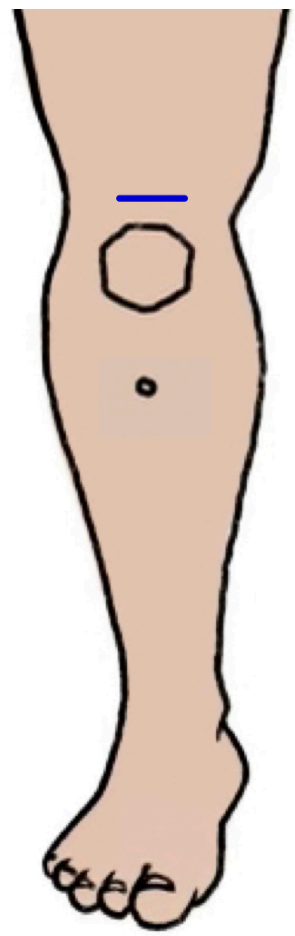


The paratenon, areolar tissue filling the space between the patellar tendon and its sheath, is bluntly dissected from the subcutaneous tissue exposing the tendon (McAllister and Wu 2012). The prepatellar bursa is then reflected and the central 1/3 or 10 mm of the patellar tendon is cut using a scalpel (McAllister and Wu 2012). A scalpel is then used to mark the bone cuts on the tibial tubercle and patella which will be used as bone plugs for the graft (McAllister and Wu 2012). An oscillating saw is then used to cut the bone aiming for a trapezoid-shaped bone plug from the tibial tubercle and a triangular-shaped bone plug from the patella (McAllister and Wu 2012). The graft is carefully dissected from the underlying adipose tissue and placed on the back table for preparation (McAllister and Wu 2012).

Quadriceps Tendon Graft

The use of the QT for ACLr was first performed by Campbell in 1935. Since then, the BPTB and HT graft have become more popular choices. Because of this the QT graft has received poor recognition and lacks standard protocols due the variability in using a bone plug or not. But recent evidence has suggested that a flat QT graft without a bone block may more closely mimic the native ACL (Fink et al. 2020). For this reason, I have chosen to focus on this surgical technique. The QT graft uses similar preoperative procedures seen in both the BPTB and HT grafts. Following clinical testing of the ACL under general anesthesia, a routine diagnostic arthroscopy is performed to confirm an ACL rupture and address and additional intra-articular pathologies like a meniscal tear (Lee et al. 2004; Geib et al. 2009). The patient’s knee is then bent to approximately 80-90 degrees of flexion to maintain tension in the QT (Lee et al. 2004; Diermeier et al. 2020). The main incision for graft harvesting is made transversely one cm proximal to the superior/proximal border or pole of the patella spanning approximately two to three cm **(illustration 3)** (Fink et al. 2020; Diermeier et al. 2020). Once the bursal layer has been dissected exposing the QT, a tendon knife with two parallel blades (10 mm width) is advanced to 7 cm (Fink et al. 2020). A tendon separator is then set to a depth of 5 mm and is advanced proximally 7 cm (Fink et al. 2020). Following this a quadriceps tendon cutter is placed one to two cm proximal to the superior border of the patella and advanced proximally up the graft until the desired length of the graft is reached (Fink et al. 2020). Then the graft is reflected distally out of the incision site and cut away from its attachment to the patella (Fink et al. 2020). Following the preparation of the QT, two standard arthroscope portals are made. First, an arthroscopic portal is made lateral to the patellar tendon at the level of the patellar apex which is used to place the second portal (Fink et al. 2020). The second portal is then made on the medial side of the patellar tendon at the same level as the lateral portal but just slightly

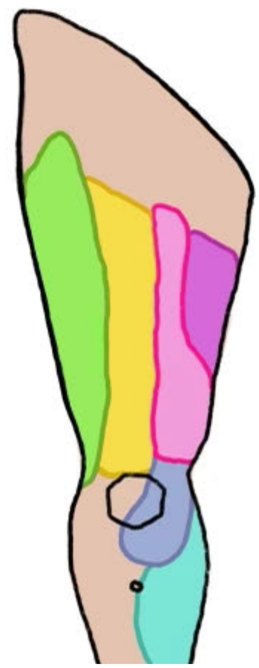
Illustration 3: QT graft main skin incision



more distal (Fink et al. 2020). This portal is then used for the remainder for the surgery to drill the femoral tunnel and place the QT graft (Fink et al. 2020).

SENSORY LOSS FOLLOWING ACL RECONSTRUCTION

Illustration 4: Cutaneous innervation map, LFCN in green, AFCN in yellow, MFCN in pink, obturator nerve in purple, IPBSN in blue, and SBSN in light blue.



Using the peripheral cutaneous nerve distribution of the leg the three different graft type incisions can be mapped to regions innervated by distinct nerves/nerve branches **(illustration 4).** The BPTB and HT graft main incision sites are within a region innervated by two branches of the saphenous nerve, the sartorial branch of the saphenous (SBSN), and the infrapatellar branch of the saphenous nerve (IPBSN) (Ruffilli et al. 2016). Due to the superior or proximal incision site of the QT graft, it is performed in a region innervated by the AFCN, MFCN, and LFCN.

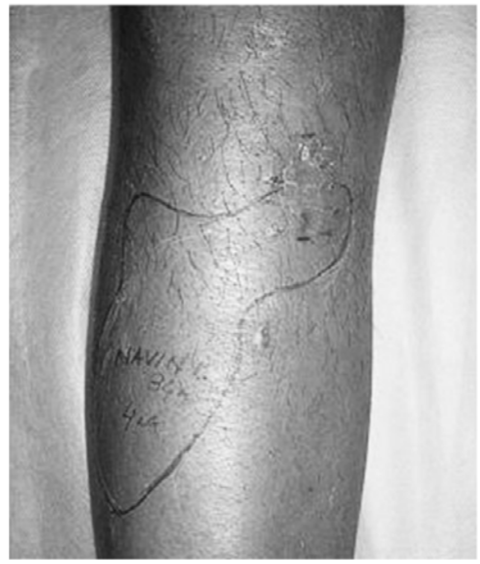
Hamstring Tendon Graft

Damage to the IPBSN has been documented during total knee replacements, tibial nailing, open meniscectomy, open and arthroscopic repairs. But damage during tendon harvesting for ACL grafts has been well reported and is one of the highest non-functional post-operate complications (Sabat and Kumar 2013; Trescot, Brown, and Karl 2013). With the emersion of minimally invasive surgical techniques the rate of IPBSN injury is even worse with anesthesia rates reported in a systematic review by Ruffilli et al. (2016) ranging between 12% and 84 % in several case series compared to open techniques with injury rates between 21% to 69% (Sanders et al. 2007). Clinically damage to the IPBSN presents with numbness, dysesthesia, and pain which is not well localized and is generally spontaneous on the anteromedial region of skin inferior to the patella (Trescot, Brown, and Karl 2013). These complications can lead to several degrees of discomfort ranging from chronic pain to slightly altered sensations (Kalthur et al. 2014).

Damage to the IPBSN and the subsequent numbness seems to be directly related to the incision sites used during HT graft harvesting as the IPBSN travels obliquely in a superior to inferior direction in the area of the incision (Ruffilli et al. 2016). It has been suggested that an oblique or horizontal incision compared to the standard vertical incisions may spare the IPBSN. While damage to the SBSN is thought to be related to the passage of the tendon stripper during the harvesting of the gracilis and semitendinosus tendons due to the nerve emerging from the adductor canal and traveling superficial to the pes anserine tendons to the lower leg (Ruffilli et al. 2016). In a study conducted by Sanders et al. (2007), a questionnaire was sent out to patients who underwent arthroscopic HT graft ACLr using a vertical incision. This included a diagram of the cutaneous innervation regions of the IPBSN and the SBSN which instructed the participants to mark the location of any numbness or paresthesia that occurred following the procedure and how significant the effect of the injury was on daily living (Sanders et al. 2007). Out of the 62 respondents, 46 or 74% reported some type of sensory disturbance following the procedure, with isolated SBSN disturbance occurring in 14 or 23% patients. Isolated IPBSN disturbances occurred in 12 or 19%, and the remaining 20 or 32% reported combined SBSN and IPBSN disturbances (Sanders et al. 2007). Out of the 74% of patients who reported sensory disturbances, 20% had improvement over 6 months and only 6.5% of the total 74% reported the disturbances significantly impacted day-to-day activities (Sanders et al. 2007).

In a randomized controlled prospective study by Sabat & Kumar (2013), 152 patients who underwent ACLr using a HT were randomly assigned into three groups, 40 patients each. Each group was assigned one of the three different HT graft incisions, vertical, horizontal (transverse), or oblique, and were performed by a single surgeon (V.K.) (Sabat and Kumar 2013). All incisions were made over the pes anserinus and carried to a length of four cm in the direction of the assigned incision. All patients were evaluated postoperatively for sensory loss using a pinprick examination at 6 weeks, three months, and 6 months (Sabat and Kumar 2013). The pinprick examination was used to map the locations of sensory changes around the incision site which were marked on the skin and then transferred to sheet paper to calculate the area of sensory loss (Sabat and Kumar 2013). Sabat & Kumar (2013) found that at 6 months the rate of persistent sensory loss in the vertical incision groups was 79% (30/38) which was found to be significantly higher ($p = 0.001$) than the horizontal and oblique incisions groups which were 43.2% (16/37) and 37.8% (14/37) respectively. When comparing the three groups at 6 months the injury rate to the IPBSN was significantly higher ($p = 0.000$) in the vertical incision group at 76.3% (29/38) compared to the horizontal incision at 35.1% (13/37) and the oblique incision at 32.4% (12/37) (Sabat and Kumar 2013). They also found that the injury rate to the SBSN was not significant between the three groups which supports the hypothesis that damage to this nerve is caused by the tendon stripper (Sabat and Kumar 2013). The results of the pin prick examination followed the rates of persistent sensory

Figure 1: Area of sensory deficits following a vertical skin incision. Taken from Sabat and Kumar (2013)



loss with the vertical incision group having the largest area of sensory, followed by the horizontal and oblique incision groups (Sabat and Kumar 2013). In the majority of the patients, the area of sensory loss was located over the distal portion of the incision and extending laterally and distal to the tibial tuberosity **(figure 1)** (Sabat and Kumar 2013). This area of sensory loss matches the distribution of the IPBSN and in many cases also includes the anterolateral leg which is not supplied by the SBSN (Sabat and Kumar 2013). This also suggests, that the LFCN may not contribute significantly to the “patellar plexus”. Over the three follow-up visits, the area of sensory loss slowly diminished from distal to proximal with the majority of the patients only having persistent sensory loss over the incision site (Sabat and Kumar 2013).

A systematic review conducted by Ruffilli et al. (2016) identified articles which compared sensory disturbances between different incision types. Three of the articles compared sensory disturbances caused by IPBSN damage between vertical and oblique incisions. Darestani et al. (2013) found that the IPBSN was damaged in 33% of the producers using a vertical incision compared to 13% with an oblique incision but the results were without significance (Ruffilli et al. 2016). Luo et al. (2007) reported incidence of IPBSN damage in 65.7% of patients who received a vertical incision versus the oblique incision which was found to be 24% (Ruffilli et al. 2016). Papastergiou et al (2006) investigated vertical incisions compared to oblique found the incidence of IPBSN damage in 40% and 18% respectively (Ruffilli et al. 2016). Conversely, Kiaergard et al. (2008) reported similar incidences of IPBSN damage between vertical and oblique incisions both at 84% (Ruffilli et al. 2016) The finding of the systemic reviews and other current literature suggest that one incision is not superior over the other in reducing iatrogenic damage to the IPBSN.

Bone-Patellar Tendon-Bone Graft

Historically the BPTB has been the gold-standard for many orthopedic surgeons and is still a popular graft choice in high-level athletes (Hurley et al. 2018). The BPTB has excellent strength, is easy to harvest, and is a consistent size across patients. Because a bone plug is taken form the tibial tuberosity and patella, the graft is associated with increased risk for harvest-site complications, patellar tendon ruptures, and long-term risk of osteoarthritis (McAllister and Wu 2012; Hurley et al. 2018). Persistent donor-site morbidity, such as tenderness, anterior knee pain, disturbance in anterior knee sensitivity, and the difficulty in kneeling or knee-walking, are reported following surgery in 40% to 60% of patients (Kartus, Movin, and Karlsson 2001; Dai et al. 2021). Harvesting the central 1/3 of the patellar tendon may cause scar tissue to form and result in kneeling pain following ALCr (Lund et al. 2014). Haviv et al. (2017) included 60 patients in a study investigating the prevalence and sensory nerve injuries in BPTB harvesting for ACLr. At the 6-month follow-up, postoperative sensory disturbances were reported in 46 (77%) of the patients with 42 of the 46 patients reporting the region of disturbances lateral to the main incisions site (Haviv et al. 2017). This again suggests, that the LFCN may not contribute to cutaneous innervation distal to the knee joint line. Similar to the HT graft, the sensory disturbances in the anterior knee are related to damage of the IPBSN. Mouarbes et al. (2020), demonstrated this reporting that in all 18 cadaveric specimens either two (12 specimens) or three branches (6 specimens) trunks of the IPBSN were located in the subcutaneous tissue deep the main incision site.

Quadriceps Tendon Graft

The QT graft for ACLr is the least studied autograft but may be the best choice for ACLr (Lund et al. 2014). The QT autograft has tremendous benefits over other graft types such as preservation of hamstring function and a reduction in the incidence of anterior knee pain which is a common complication with the BPTB and HT autografts (Lund et al. 2014). The QT graft uses a transverse or horizontally oriented two to three cm long incision in a region of skin proximal to the superior border of the patella. Modern peripheral cutaneous nerve distribution maps show this region of skin is innervated by anterior cutaneous branches of the femoral nerve Lund et al. (2014) used a randomized controlled study to compare patient outcomes, such as knee stability, kneeling pain, harvest site pain, sensory loss, and subjective clinical outcomes following ACLr between QT and BPTB autograft. 51 patients were randomly assigned, with 25 patients assigned to QT autograft ACLr and 25 patients assigned to BPTB autograft ACLr (Lund et al. 2014). Follow-up evaluations were performed at one and two years post-operation by an independent physiotherapist (Lund et al. 2014). They found that knee laxity showed a difference of 0.8 ± 1.7 mm SD in the BPTB group and 1.1 ± 1.4 mm SD in the QT group (p=0.65) (Lund et al. 2014). Lund et al. (2014) did find a significant difference in anterior kneeling pain and harvest site pain between the two groups. At the 1-year follow up 34% of patients who underwent ACLr using a BPTB autograft reported the two lowest scores for the knee walking test (impossible or difficult) compared with only 5% of the QT group reporting similar kneeling difficulties (p=0.03) (Lund et al. 2014). Also, at the 1-year follow up, harvest site pain was significantly less in patients who received QT grafts at 0% compared to 30% in the BPTB group (p=0.02) (Lund et al. 2014). Sensory loss occurred in 73% of patients in the BPTB group with only 48% of the QT group reporting sensory loss (p=<0.05) along with the area of sensory loss being $62cm^2$ in the BPTB group and $39cm^2$ in the QT group (p=<0.01) (Lund et al. 2014). The results of this study support previous findings by Kartus et al. (1999) that the median incision used for harvesting the central third of the patellar tendon leads to a greater risk of damage to the IPBSN and can result in increased kneeling pain and sensory loss (Lund et al. 2014). Due to the location of the incision used for harvesting the QT, only the small incisions medial to the tibial tuberosity that is used for tibial drilling is within the region of the IPBSN which could be the reason behind the reduced sensory loss (Lund et al. 2014).

Hopton et al. (2004) recruited 91 patients, 66 females, and 25 males, during follow-up visits to investigate the rate of skin numbness following the operation (Hopton, Tommichan, and Howell 2004). Along with this they also attempted to define the anatomy of the cutaneous nerves via dissection of four donor knees (Hopton, Tommichan, and Howell 2004). Hopton et al. (2004) found that 86% of knees had a lateral numb area but only 60% of these patients reported numbness (p=<0.05) (Hopton, Tommichan, and Howell 2004). Of this 60% group, 62% had improvement over the 8-month follow-up with 20% expressing concern about the numbness and 4.5% stating a significant problem that affected daily activities of living (Hopton, Tommichan, and Howell 2004). Interesting, only 82% of female knees had numbness with male knees reporting numbness in 97% of patients (Hopton, Tommichan, and Howell 2004). The findings of the cadaveric study support this rate of numbness due to the anatomy of the main branches of the MFCN and AFCN sweeping oblique across the incision site, with injury to the saphenous nerve and IPBSN being unavoidable due to their proximity to the tibial tuberosity (Hopton, Tommichan, and Howell 2004). An anatomical study in five donors conducted by Le Corroller et al. (2011) added to the results of Hopton et al. (2004) finding that infrapatellar branches from the MFCN were located within a region between 55 mm from the medial border of the patella and 44 mm from the medial border of the patellar tendon (Hu et al. 2015). Mourabes et al. (2020) found that the mean measured area of hypoesthesia was significantly higher (p=<0.0001) in BPTB autograft group ($88.2 \pm 57\ cm^2$) compared to the QT autograft group ($8.7 \pm 5.1 cm^2$). Mourabes et al. (2020) also found the area of hypoesthesia was significantly higher (p=<0.0001) in HT autograft group ($70.3 \pm 77.1\ cm^2$) compared to the QT autograft group ($8.7 \pm 5.1 cm^2$).

Current surgical innervation to the knee is performed with little regard to the cutaneous nerves despite the evidence that nerve injury often leads to painful neuroma or reflex sympathetic dystrophy (Hurley et al. 2018). There is a lack of literature on the extent of sensory loss in patients treated with QT autografts and even less literature investigating the femoral cutaneous branches within this region (Mouarbes et al. 2020). The majority of cadaveric studies investigating the anatomy and branching of the AFCN, MFCN, IFCN, and LFCN solely focus on the relation to femoroacetabular joint operations as these nerves are commonly damaged (Surucu et al. 1997; Ropars et al. 2009). Only Nogler et al. (2001) and Hopton et al. (2004) studied the femoral cutaneous nerves as they relate to the knee joint and both of these studies lacked large sample sizes, 20 and four knees respectively, and had poor methodology resulting in poor descriptions of these nerves.

DEVELOPMENT & MIGRATION OF PERIPHERAL CUTANEOUS NERVES

The peripheral nervous system (PNS) is composed of afferent somatosensory fibers that transmit sensory information from the soma to the central nervous system (CNS) and efferent somatomotor fibers that innervate skeletal muscle (Catala and Kubis 2013). Afferent fibers mediate/transmit proprioception and mechanoreception from receptors located on the peripheral limb of neurons in the dorsal root ganglion. These receptors produce action potentials, that travel on axons, which comprise a peripheral cutaneous nerve, toward the spinal cord. Once in the spinal cord, these fibers ascend as part of the dorsal column-medial lemniscus pathway, synapsing along their journey in the medulla before the tract continues to somatosensory cortices in the brain.

The afferent fibers of the PNS originate from neurons that develop from neural crest cells, which is distinct from the efferent fibers that develop from the neural tube (Koop and Tadi 2022; Catala and Kubis 2013). Following the formation of the trilaminar disc, the notochord induces the ectoderm to thicken, forming neuroectoderm and eventually the neural plate. As the plate continues to expand, an axial fold, the neural groove, develops producing folds on either side. On the lateral edges of the folds, where neuroectoderm merges with surface ectoderm, a ridge-like, neural crest forms. As the neural groove continues to deepen, the lateral edges are brought closer together and begin to fuse in the occipitocervical region. After the fusion is complete, a hollow tube, the neural tube is formed. Following fusion, the neural crests, in the roof of the neural tube, undergo an epithelial-mesenchymal transition which allows them to separate from the tube and migrate into the paraxial mesoderm layer as neural crest cells (Catala and Kubis

2013). These cells can then migrate along different pathways to give rise to a number of different structures throughout the body. The spinal neural crest cells that migrate along the intra-somitic pathway will generate DRG which contains the soma of sensory cells and their satellite cells (Catala and Kubis 2013). However, this migration can only begin once a somite has differentiated into its dorsal dermomyotome and ventral sclerotome (Catala and Kubis 2013). Molecular elements, involved in the permeability of somites, guide these cells to form the segmented roots of the PNS and prevent mixing (Catala and Kubis 2013). Once the neural crest cells have reached their final location, axons begin to develop at a differentiation on their distal end called the growth cone (Catala and Kubis 2013). The growth cone relies on the presence of chemoattractant and chemorepellent factors in the extracellular matrix to guide the growth of the axon to reach its region of innervation (Catala and Kubis 2013).

ANATOMY OF THE FEMORAL CUTANEOUS AND SAPHENOUS NERVES

Anterior and Medial Femoral Cutaneous Nerves

The femoral nerve or anterior crural nerve is the largest nerve arising from the lumbar plexus. The femoral nerve is formed by the dorsal divisions of the ventral rami from spinal nerves L2 to L4 (Refai, Black, and Tadi 2022). The femoral nerve has both motor and sensory functions, playing a major role in sensory innervation and motor control of the lower extremities. Within the pelvic cavity spinal nerves L2-L4 travel through the psoas major and combine to emerge as a single nerve, the femoral nerve, at the distal portion of the muscles lateral border (Gray 1974). The femoral nerve travels towards the inguinal ligament between the psoas major and the iliacus and eventually crosses under the inguinal ligament to enter the femoral triangle. The femoral triangle is formed by the inguinal ligament superiorly, sartorius laterally, and adductor longus medially (Refai, Black, and Tadi 2022). The femoral nerve is located lateral to the femoral sheath, quickly dividing into an anterior and posterior division which are split by the lateral femoral cutaneous artery (Gray 1974; Refai, Black, and Tadi 2022). MFCN and AFCN both arise from the anterior division of the femoral nerve with the saphenous nerve branching from the posterior division within the triangle.

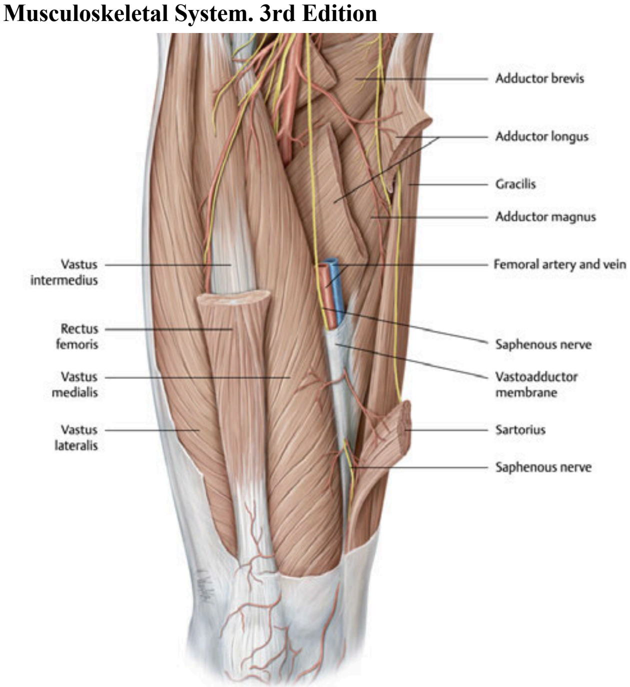
The AFCN travels obliquely across the femoral artery from lateral to medial where it divides into an anterior and posterior branch (Gray 1974). The anterior branch of the AFCN travels distally on top of the sartorius muscle and eventually pierces the fascia lata in the lower third of the thigh dividing into two branches (Gray 1974; Kampitak et al. 2021). These two branches supply the skin as distal as the medial side of the knee with the other branch crossing the lateral side of the patella (Gray 1974). The anterior branch of the AFCN descends along the lateral border of the sartorius muscle towards the knee where it pierces the fascia lata to give off several cutaneous branches some of which communicate the saphenous nerve contributing to the "patellar plexus" (Gray 1974). One of these branches passes through the saphenous opening, the second becomes subcutaneous around the middle third of the thigh and the last branch pierces the fascia in the lower third of the thigh. The posterior division of the AFCN continues and at the lower portion of the adductor longus muscle joins a plexus of nerves formed by branches of the saphenous and obturator nerves (Gray 1974). The MFCN has a variable course and branching pattern but generally travels anteromedially to the femoral artery to pierce the fascia lata about three inches distal to the inguinal ligament (Gray 1974; Kampitak et al. 2021). Upon piercing the fascia, the MFCN travels and then divides into two branches which descend along the anteromedial portion of the thigh (Gray 1974; Kampitak et al. 2021). Horner and Dellon (1994) performed an anatomical dissection of fourth-five fresh human thigh/knee specimens from 25 donors to investigate the cutaneous innervation pattern of the knee joint. They found that the MFCN constantly traveled and terminated in the superficial layer of adipose tissue (Horner and Dellon 1994). In 39.1% of the specimens, Horner and Dellon (1994) observed the MFCN traveling superficially to the sartorius muscle but also reported in 30.4% of their specimens the MFCN traveled in the adductor canal eventually perforating the sartorius muscle (Horner and Dellon 1994). In the remaining 30.4% of the specimens, the MFCN traveled through the adductor canal, similarly to the saphenous nerve, and exited deep to the sartorius (Horner and Dellon

1994). Ultimately, the MFCN supplies the skin of the anteromedial thigh as distal as the knee where it communicates with the AFCN and branches of the saphenous nerve forming a portion of the "patellar plexus" (Gray 1974; Kampitak et al. 2021; Horner and Dellon 1994).

Saphenous Nerve

The saphenous nerve is composed of nerve fibers from L3 and L4 spinal levels originating from the posterior division of femoral nerve just distal to the inguinal ligament. The saphenous nerve is a purely sensory nerve composed of two terminal branches, the infrapatellar branch of the saphenous nerve (IPBSN) and sartorial branch of the saphenous nerve (SBSN). The IPBSN provides sensory innervation to the anteromedial aspect of the knee between the inferior margin of the patella to the anterior inferior aspect of the knee capsule and the SBSN provides sensory innervation to the anteromedial leg and foot (Trescot, Brown, and Karl 2013; Ruffilli et al. 2016). After branching from the femoral nerve, the saphenous nerve then travels distally with the femoral vasculature into the adductor canal **(figure 2)**. Eventually, the saphenous nerve pierces the vasto-adductor membrane with the

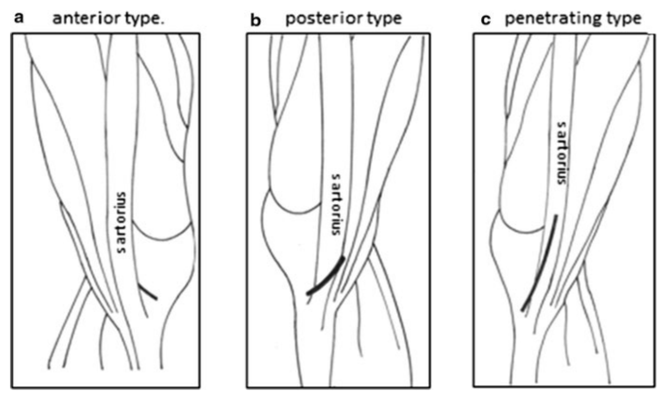
Figure 2: Saphenous nerve anatomy. Adopted from THIEME Atlas of Anatomy, Volume 1: General Anatomy and Musculoskeletal System. 3rd Edition



descending genicular branch of the femoral artery (Trescot, Brown, and Karl 2013). Upon exiting the adductor canal medially the saphenous nerve divides into its two terminal branches the SBSN and IPBSN (Sabat and Kumar 2013). After dividing these nerves take a variable course to reach their cutaneous innervation region. Arthornthurasook (1988) studied the relationship of the IPBSN to the sartorius muscle in 20 cadavers and found that in 62% of the cadaver limbs studied the IPBSN became superficial along the posterior border of the sartorius and crossed anteriorly over the muscle as it traveled to the anterior knee (Trescot, Brown, and Karl 2013). In the remaining cadaver's, Arthornthurasook (1988) found that in 22% the nerve penetrated through the sartorius, 14% the nerve traveled in parallel and posterior to the sartorius, and in only 3% the nerve emerged along the anterior border of the sartorius. (Trescot, Brown, and Karl 2013). The most interesting finding in Arthornthurasook study, was that the IPBSN was only identical between the left and right knee in half of the cadavers (Trescot, Brown, and Karl 2013). Years later, Arthornthurasook and Gaew-Im (1999) performed a detailed follow up examination of the IPBSN and the SBSN on 60 specimens (Trescot, Brown, and Karl 2013). They found that the SBSN reliably crosses the gracilis tendon at the posteromedial joint line of the knee and then becomes superficial between the gracilis tendon and the sartorius muscle (Trescot, Brown, and Karl 2013). After emerging between the gracilis tendon and sartorius, the SBSN continues subcutaneously to provide sensory innervation to the anteromedial leg and foot (Trescot, Brown, and Karl 2013). As the SBSN travels superficial it and some of its branches travel in the region of HT graft incisions and other procedures involving incisions on the postero-medial knee (Trescot, Brown, and Karl 2013).

Additionally, Kalthur et al. (2014) preformed anatomical dissection of 32 lower limbs from 16 cadavers to study the relationship of the IPBSN to the sartorius. They classified the IPBSN relative to where the nerve crosses the border of the sartorius as anterior or posterior type, and whether the nerve pierces the muscle which is classified as penetrating type (Kalthur et al. 2014) **(figure 3)**. They found that the IPBSN was classified as an anterior type in 68.7%, penetrating type at 28.1%, and finally the posterior type occurring in 3.1% of the 32 specimens (Kalthur et al. 2014). When comparing left and right sections, the anterior type was almost similar, occurring in 62.5% or 10 out of 16 on the right, and 75% or 12 out of 16 on the left (Kalthur et al. 2014). Interestingly the penetrating type was much more common on the right compared to the left, occurring 37.5% of the time, or six out of 16, and 18.7%, or three out of 16 respectively with the posterior type occurring only on the left in one out of 16 (Kalthur et al. 2014). Horner and Dellon (1994) investigated the location of where the IPBSN and its branches divide from the main saphenous nerve as well as the relationship to the sartorius, femoral nerve branches and obturator nerve branches (Trescot, Brown, and Karl 2013). They found that the IPBSN divides from the main trunk of the saphenous nerve in

Figure 3: IPBSN classification based on relationship to the sartorius. Taken from Kalthur et al. (2014)



three general regions along the thigh. In the proximal or superior thigh the IPBSN divides 18% of the time, in the mid-thigh 59% of the time, and in the distal or inferior thigh 24% of the time (Trescot, Brown, and Karl 2013). While studying the saphenous terminal branches location in relation to the sartorius, Horner and Dellon (1994) classified that in most individuals, two or more branches exit the adductor canal proximal to the knee joint (Trescot, Brown, and Karl 2013). The more anterior branch, the IPBSN, crosses the knee medial to lateral supplying cutaneous innervation to the region distal to the patella and also contributing to some sensory innervation of the anterior inferior joint capsule (Trescot, Brown, and Karl 2013). The more posterior branch, the SBSN, is the continuation of the main saphenous nerve as it travels distally medial and deep to the sartorius before piercing the fascia between the sartorius and gracilis tendon (Ruffilli et al. 2016; Trescot, Brown, and Karl 2013). The nerve branch then becomes subcutaneous and continues distally providing sensory innervation for the medial aspect of the leg and ankle (Ruffilli et al. 2016).

The course of the IPBSN is variable between individuals and has even been shown to vary between left and right knees (Kalthur et al. 2014). Kalthur et al. (2014) measured the distance between the main trunk of the IPBSN to the medial margin or border of the patella using digital calipers (Kalthur et al. 2014). The distance of the IPBSN trunk to the medial border of the patella was found to fall within a range of 4-9mm, with the right side ranging from 4-9mm and the left falling between four to 7mm (Kalthur et al. 2014). Within these ranges, Kalthur et al. found that the nerve trunk was located between five and 5.9mm from the patella in 40.6% of the donors followed by a range of four to 4.9 mm in 31.2% (Kalthur et al. 2014). Mochida and Kikuchi (1995) examined the IPBSN, focusing on the location of where the IPBSN crosses the proximal edge of the tibia (Trescot, Brown, and Karl 2013). Based on their dissection of the nerve, they were able to find 2 predominant patterns with constant relationships between the patellar ligament (or tendon) and the tibial plateau (Trescot, Brown, and Karl 2013). The more common pattern, occurring in 68% of the cadavers, found that the IPBSN crosses the knee joint medial to the medial margin of the patellar ligament (Trescot, Brown, and Karl 2013). The other IPBSN pattern, occurring in 32% of the cadavers, crosses the patellar ligament proximal or superior to the knee joint (Trescot, Brown, and Karl 2013). Based on their measurements and findings they were able to identify a "safe zone" between the IPBSN and the medial margin of the patellar ligament (Trescot, Brown, and Karl 2013). Two years later Ebraheim and Mekhail (1997) studied 28 specimens, 15 males and 13 females, confirming the "safe zones" identified by Mochida and Kikuchi (1995) (Trescot, Brown, and Karl 2013). Three years following this publication, Mochida and Kikuchi along with Tifford et al. (2000) observed in 20 specimens substantial changes in the location of the IPBSN based on the position of the knee, which indicated that the "safe zone" identified by Mochida and Kikuchi (1995) can change (Trescot, Brown, and Karl 2013).

Kalthur et al. (2014) counted the number of terminal branches and the anatomical location of termination using the patella and tibial tuberosity as bony landmarks. As expected, the terminal branches of the IPBSN were variable with one to three branches being identified. The single branching pattern was observed in 40.6% of the donors with two branches occurring in 34.3% and three branches occurring in 25% (Kalthur et al. 2014). The location of termination for these branches in relation to the patella and the tibial tuberosity also varied. Of the 13 out of 32 donors that had a single branch pattern, 11 had a single nerve branch that passed between the patella and tibial tuberosity, one donor had a branch at apex of the patella and the last donor had a branch that ran below the tibial tuberosity (Kalthur et al. 2014). Within the group where two branches were observed, in 11 out of 32 donors, the two nerve branches traveled between the patella and tibial tuberosity in four donors and traveled at the level of the tibial tuberosity in three donors (Kalthur et al. 2014). In the four remaining donors, the two branches of the IPBSN traveled in different locations. In one donor the more superior branch traveled at the level of the patella and the inferior branch traveled between the patella and tibial tuberosity (Kalthur et al. 2014). In two of the donors the superior branch traveled at the level of the patella while the inferior branch traveled between the patella and tibial tuberosity and in the last donor the superior branch traveled between the patella and tibial tuberosity with the inferior branch traveling to the tibial tuberosity (Kalthur et al. 2014). Lastly, within the eight out of 32 donors of whom three branches of the IPBSN were observed, four main patterns were found. In two of the donors all three branches traveled between the patella and tibial tuberosity, one donor had all three branches travel at the level of the tibial tuberosity with the remaining five having variability (Kalthur et al. 2014). In one donor, a single branch was observed at the patella with the remaining two branches traveling between the patella and tibial tuberosity, two donors had one branch between the patella and tibial tuberosity with the remained traveling to the tibial tuberosity and the finial donor had two branches between the patella and tibial tuberosity with only a single branch at the level of the tibial tuberosity (Kalthur et al. 2014).

Lateral Femoral Cutaneous Nerve

The external or lateral femoral cutaneous nerve (LFCN) is formed from dorsal branches of ventral rami of the L2 and L3 spinal levels. The LFCN emerges from approximately the middle portion of the outer or lateral border of the psoas major muscle and travels obliquely on top of the iliacus muscle towards the inguinal or Poupart's ligament (Gray 1974; Surucu et al. 1997). The LFCN crosses under the inguinal ligament and superficial to the sartorius muscle into the proximal anterior thigh, medial to the ASIS. The LFCN then divides into two main branches, an anterior/femoral branch and a posterior/gluteal branch (Gray 1974; Ropars et al. 2009). The anterior branch of the LFCN then travels for approximately four inches in an aponeurotic canal formed by the fascia lata until the branch becomes superficial (Gray 1974). Once superficial, the nerve divides and innervates the skin along the anterolateral portions of the thigh as far distal as the knee and occasionally communicates with the IPBSN contributing to the "patellar plexus" (Gray 1974; Kampitak et al. 2021; Surucu et al. 1997). Additionally, Rubin et al. (2016) classified the LFCN in the anterior thigh into three main types based on the dominant branching pattern. A sartorius-type is classified as having a dominant anterior branch traveling on the lateral border of the sartorius branching into the anterior thigh (Thaler et al. 2020). A posterior-type is classified as a large posterior brancher equal to or thicker than the anterior branch traveling laterally (Thaler et al. 2020). Lastly the fan-type was classified as multiple nerve branches of equal thickness in the anterolateral thigh, traveling on the lateral border of the sartorius and posteriorly (Thaler et al. 2020).

RESEARCH PURPOSE

The goal of this thesis was to perform a very detailed map of the location of the cutaneous nerves in the anteromedial thigh/knee regions through cadaveric dissection and explore ultrasound techniques to locate the nerves. Following the dissection of the femoral cutaneous and saphenous nerves, topographic maps were generated, mapping the anatomical course of the nerves and their terminal branches to relevant anatomical landmarks. The topographic maps were then normalized and aligned to inguinal ligament and sartorius in the anterior thigh, and to the patella, tibial tuberosity (TT), and sartorius at the knee joint in order to be able to compare the anatomical branching patterns across specimens. These structures serve as both anatomical and clinical landmarks for diagnostic, surgical procedures and/or ultrasound imaging (Longino and Giffin 2012; McAllister and Wu 2012; Lee et al. 2004; Geib et al. 2009; Diermeier et al. 2020). The major limitation to the gold standard of cadaveric dissection is the difficulty gathering the three-dimensional aspect of the tissue planes the nerves travel due to tissue removal during dissection. To help further define these planes we validated current ultrasound (US) techniques by locating the femoral cutaneous nerves as they travel through the undistributed subcutaneous tissue of the cadavers. In addition, methods to increase visualization of the terminal nerve branches were also explored. Generating topographic maps utilizing a combination of cadaveric dissection, and US imaging have the potential to advance our understanding of diverse knee innervation patterns and thus impact surgical interventions related to the knee joint. Topographic techniques of positional frequency and spatial heat mapping for the femoral cutaneous nerves will help surgeons guide skin incisions during ACL reconstructive surgery aiding in potential reduction of iatrogenic nerve damage and subsequent sensory deficit. In addition, a better understanding of the anatomy of the femoral cutaneous nerves on US can help surgeons locate the nerves pre-operatively and avoid them during skin incisions.

METHODS

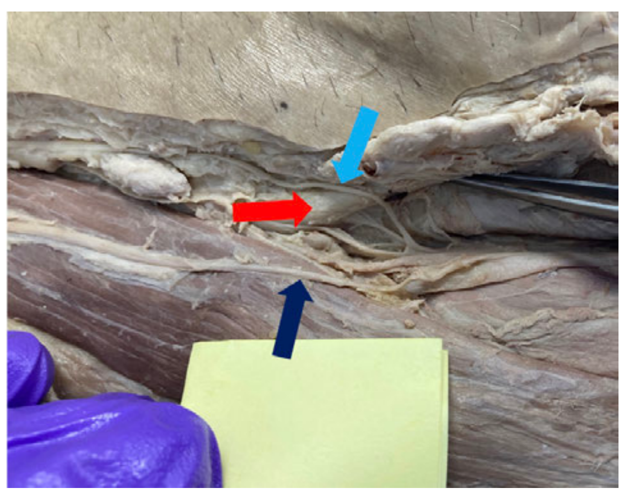
Cadaveric Dissection

Two embalmed cadavers' part of Boston University Chobanian & Avedisian School of Medicine pre-clinical anatomical courses were dissected in exquisite detail over six months, yielding two lower extremities. Each specimen's thorax, abdomen, and pelvis were dissected with the lower extremities intact. All donors were biological females with no major signs of surgery or trauma to their lower extremities. All cadavers were placed in the supine position. Before dissection, the ASIS, pubic tubercle, patella, and tibial tuberosity were palpated and marked on the skin. The length of the inguinal ligament (from ASIS to pubic tubercle) was measured, and the midpoint was marked. The distance from the midpoint of the inguinal ligament to the base (superior border) of the patella was then marked. Additionally, the size of the patella was measured and the distance from the apex (inferior border) of the patella to the TT was also measured. For the LFCN the following parameters were identified during the dissection: 1) Intrapelvic course and divisions, 2) relationship to ASIS entering the anterior thigh, 3) classification of the branching pattern in the anterior thigh according to Rudin et al. (2016), 4) relationship to the sartorius, and 5) contribution to the "patellar plexus". The following parameters were identified during the dissection for the AFCN and MFCN: 1) Origin from the femoral nerve (division), 2) relationship to the femoral artery (MFCN only), 3) relationship to the sartorius, 4) number of trunks, and 5) contribution to the "patellar plexus". Lastly for the saphenous nerve, the following parameters were identified during the dissection: 1) Origin from the femoral nerve (division), 2) relationship to the sartorius (anterior, posterior, or penetrating types), 3) terminal branches (IPBSN and SBSN), 4) relationship to the patella and tibial tuberosity, and 5) number of trunks crossing medial to lateral.

The dissection began by locating the LFCN emerging from the mid-lateral border of the psoas major, tracing the intrapelvic course distally to determine if the division split proximal to the inguinal ligament. Blunt dissection was used to separate the connective tissue above the LFCN, leaving the inguinal ligament undisturbed. The dissection was then advanced under the inguinal ligament distally into the anterior thigh, separating the tissue superficial to the nerves. This creates an artificial plane, superficial to the nerve, that allows a probe to pass into the anterior thigh from within the pelvic cavity. Next, a skin incision was made approximately four cm inferior and two cm lateral to the ASIS in the anterolateral thigh, a safe region as the LFCNs typical course into the anterior thigh is medial to the ASIS. The incision was carried through the epidermis, dermis, and subcutaneous tissue stopping at the deep fascia of the thigh (fascia lata). Careful blunt dissection was then used to separate the superficial tissues from the fascia lata creating a plane medially towards the probe and LFCN. The dissection is kept superficial to the fascia lata to avoid damage or disturbance to the nerve, as it can enter the anterior thigh deep to the fascia lata and then pierce it or can emerge superficial to the fascia. The subcutaneous tissue was dissected to expose the trunk(s) of the LFCN while preserving the thin sheet of fascia/connective tissue covering the nerves maintaining their anatomical location. At this point the intrapelvic split of the LFCN, if present, was confirmed by pulling on both trunks and visualizing the nerves in the anterior thigh. Additionally, nerve branches identified traveling posteriorly to the gluteal region were not fully dissected. Lastly, the branching pattern of the LFCN in the anterior thigh was classified according to Rubin et al. (2016): 1) sartorius type: large anterior branches/divisions travels on the lateral border of the sartorius branching into the anterior thigh, 2) Posterior type: dominant posterior branches/divisions equal to or thicker than the anterior branch/division, and 3), Fan type: multiple nerve branches of equal thickness on in the anterolateral thigh, traveling posteriorly (Thaler et al. 2020).

The dissection continued medially in the same plane to expose the deep fascia covering the femoral triangle. Again, blunt dissection was used to separate the connective tissue above the femoral nerve, leaving the inguinal ligament undisturbed so a probe can be placed and advanced into the plane from within the pelvis. The probe was then palpated and the fascia over the probe was split to open the femoral triangle. The adipose tissue within the triangle was carefully removed starting superiorly, locating the femoral sheath first, and then working laterally to locate the femoral nerve. The remaining adipose and connective tissue are then dissected to locate the anterior and posterior divisions of the femoral nerve, which are split by the lateral femoral cutaneous artery. In this step of the study, both the AFCN and MFCN were dissected and identified, arising

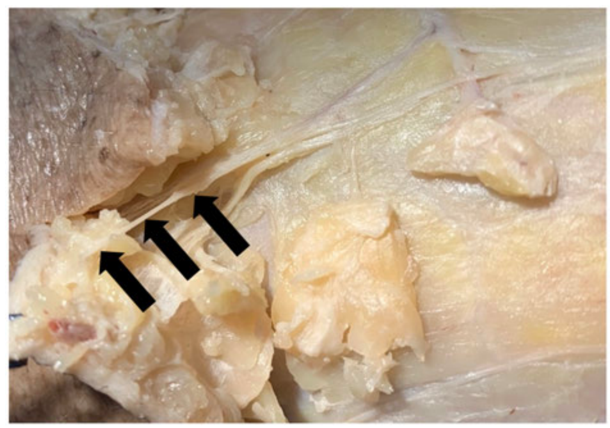
Figure 4: AFCN, dark blue arrow, MFCN, light blue arrow, and femoral artery, red arrow, exiting the femoral triangle.



from the anterior division of the femoral nerve. The AFCN was identified as traveling superficial to the sartorius, and the MFCN traveling anteromedially to the femoral artery **(figure 4).**

After locating all the main trunks of the femoral cutaneous nerves (AFCN, MFCN, and LFCN), the same dissection technique and parameters were used to follow the nerves intrafascial course distally. To maintain the nerve's anatomical location, scissors were advanced on top of the nerve, blunt dissecting the subcutaneous tissue off, leaving the fascial sheet attached. Once the tissue is separated from the nerve, the adipose tissue and skin were cut and reflected, and blunt dissection was continued. As the nerves travel distally, the thin fascial sheet was removed after the adipose tissue to help identify any branches that may have been obscured. After identifying and dissecting any branches, the nerve was glued back down maintaining its anatomical position. Lastly, as branches become fully subcutaneous, the surrounding skin/adipose tissue where the nerve could no longer be followed was cut out and glued down **(figure 5).**

Figure 5: Fully subcutaneous terminal branch



Because the saphenous nerve travels deep to the cutaneous innervation region of the MFCN, in the adductor canal, it was dissected after the femoral cutaneous nerves had all terminated and been mapped. The saphenous nerve was first located branching from the posterior division of the femoral nerve. The saphenous nerve was then dissected and followed distally as it travels deep the Sartorius. Care was taken to free the sartorius from the deep fascia allowing the muscle to be reflected and the saphenous nerve to be followed into the adductor canal. The adductor canal was then exposed by splitting the aponeurosis of the vastus medialis and blunt dissection was continued following the medial border of the sartorius. The dissection was continued distally, stopping approximately at the knee joint line where the saphenous nerve typically pierces the vasto-adductor membrane/deep fascia to exit the adductor canal and divides. The SBSN was identified as traveling vertically to the medial lower leg and the IPBSN crossing laterally towards the patellar ligament. Depending on the location of the division, the IPBSN or saphenous nerve was classified based on its relationship to the sartorius, anterior, posterior, or penetrating type. Using blunt dissection, the IPBSN was then exposed, following its oblique course towards the infrapatellar region. Lastly, the SBSN was partially dissected, to verify the presence of any branches within the cutaneous region of interest and all remining tissue in the infrapatellar region was removed.

Spatial Registration and Topographic Mapping

The thigh is roughly cylindrical, starting wider proximally at the gluteal folds and gradually narrowing distally towards the knee joint. The course of the cutaneous nerves to supply the “patellar plexus” are highly variable between specimens and travel in several different regions. The unique course and twisting of the sartorius uniquely follows the shape of the thigh, and also serves as an anatomical/clinical landmark. In order to perform analysis, the three-dimensional shape of nerves branching patterns as they travel distally down the thigh has to be mapped and flattened in a two-dimensional plane **(figure 6).** To accomplish this, the length of the sartorius muscle was measured using string temporarily attached to the medial and lateral border of the muscle from its origin at the ASIS to its insertion at the pes anserinus. The width of the sartorius, medial to the lateral border, was also measured and used to generate a flattened sartorius in Adobe Photoshop. Once the remaining landmarks were added (patella and TT), photographs of the specimens in situ and visual inspection were used to map the cutaneous nerves relative to the landmarks. A ruler was included in each picture for subsequent measurement of the nerves using an open-access photo measuring tool. This tool makes measurements on an image using an object with a known size, the ruler, for scale which allows for more precise measurement of individual branches. In addition, the femoral cutaneous and saphenous nerves were painted using the same color coding to distinguish the branches on the specimens. After the paint is dried, a section of clear plastic is draped over the lower extremity and

Figure 6: Visual representation of how the topographic maps were made.

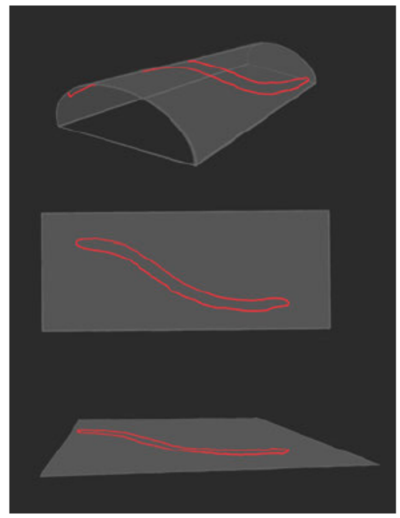


Figure 7: Plastic sheet draped over the knee with transferred branching patterns.

attached with pins. During this process care was taken to ensure the plastic was in direct contact with the tissue, following the cylindrical shape of the thigh. The nerves and landmarks were then transferred to the plastic producing a 1:1 scale copy of the innervation pattern **(figure 7).**

Ultrasound

During the course of the dissections an ancillary project was explored to further characterize the cutaneous nerves and provide a potential pre-operative technique to locate cutaneous nerves. In general, the nerves innervating the patellar region are quite difficult to visualize from the surrounding tissue on US due to their small diameter (0.5-3 mm) (Hu et al., 2015). With advancing ultrasound (US) technology, we investigated different techniques and methods to locate distal the branches of the femoral cutaneous nerves. In one cadaveric specimen, the AFCN was identified proximal in the femoral triangle and then dissected distally stopping approximately 15 cm superior to the patella. A region of subcutaneous tissue was chosen with a relatively large number of nerve branches entering undistributed subcutaneous tissue distally. The nerves were assigned letters in numerical order from medial to lateral and their depth below the skin was approximated. Next, an L12-5 US probe in the MSK general imaging mode was used to scan the undissected tissue to identify the nerves on US. Sufficient US gel was used between the probe and bag and on top of the skin. Single transverse plane images were obtained to locate the corresponding nerve branches. Using the approximated depth of the nerves, they were identified as honeycomb-like hypoechoic structures surrounded by hyperechoic connective tissue (Yeo et al., 2020). Additionally, to confirm the branches were successfully located the US probe was fanned front to back and sequential images were acquired to confirm the honeycomb-like structure was present both proximally and distally in the same approximate tissue plane. The epidermis, dermis, subcutaneous tissue, and various fascial layers were also identified on US and used to define the tissue plane the nerve is traveling in.

RESULTS

Cadaveric Dissection

In all specimens, the LFCN was located emerging from the lateral border of the psoas major muscle. In specimen two, the femoral and gluteal branches were located dividing along their intrapelvic course. The LFCN was observed entering the anterior thigh medial to the ASIS in both specimens. In the proximal thigh, the LFCN was consistently located tightly adhered to the surface of the deep fascia with some branches becoming subcutaneous before quickly terminating. According to the Rudin et al. (2016) classification we observed a fan-type branching pattern in specimen one, and a sartorius-type LFCN branching pattern in specimen two **(figure 8)**. In the proximal thigh, an anterior trunk was observed in both specimens traveling along the lateral border of the Sartorius. The more dominant anterior trunks observed in the sartorius-type pattern from specimen two, traveled significantly more distal along the lateral border of the muscle, before following the curvature of the thigh and terminating laterally. Lastly, in both specimens, no branches of the LFCN were observed extending to the tissue adjacent to the patella and supplying the "patellar plexus".

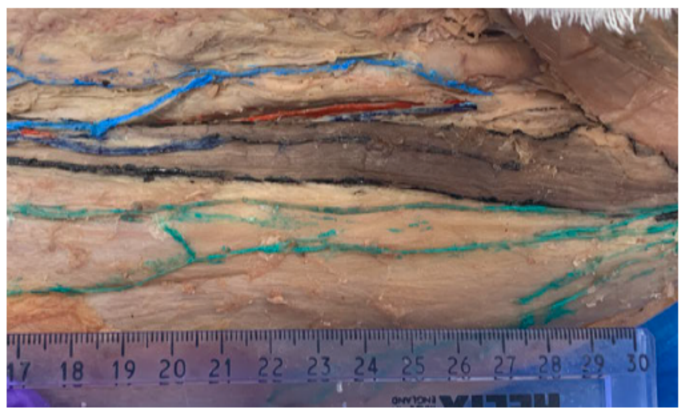
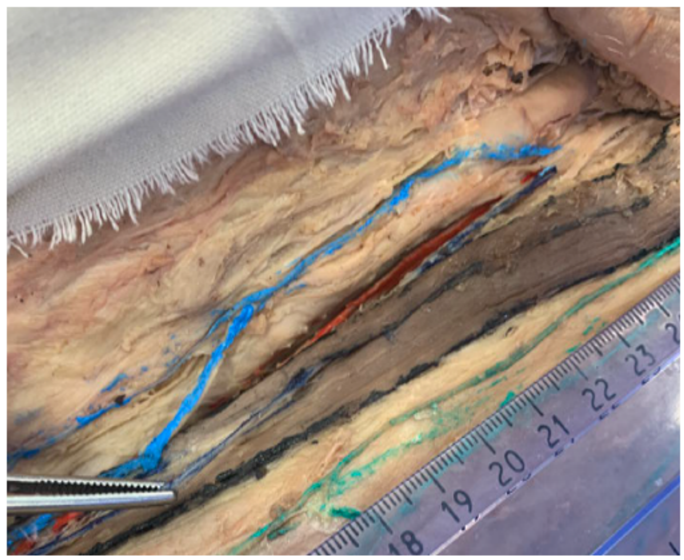


Figure 8: Lateral view of the sartorius-type LFCN branching pattern from specimen two. LFCN in green, AFCN in dark blue, and MFCN in light blue.

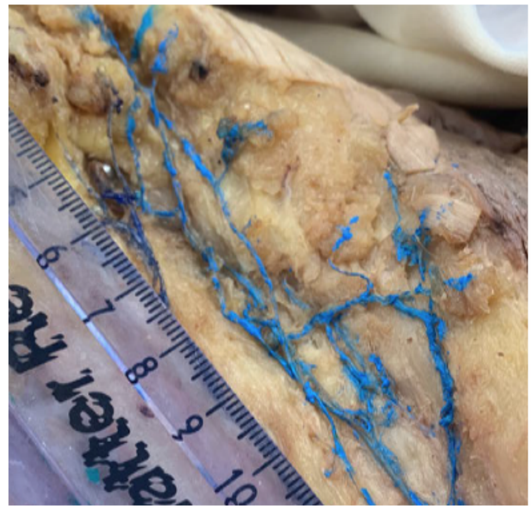
In all specimens, the femoral nerve was located lateral to the femoral artery within the femoral triangle. The AFCN was observed branching from the anterior division of the femoral nerve in all cases. In specimen one, the AFCN demonstrated a typical course, piercing the fascia lata and crossing superficial to the sartorius. In contrast, a unique anatomical variation of the AFCN was observed in specimen two, in which after branching from the anterior division, the nerve pierced and traveled through the sartorius, creating an accessory muscle slip **(figure 9)**. After traveling through the muscle, the AFCN quickly pierced the deep fascia and continued a typical course. In all specimens two main trunks (anterior and posterior branches) were identified dividing distal to the sartorius. Overall, in both specimens, the anterior branches were slightly larger and provided more terminal branches compared to the posterior branches. Additionally, in specimen one, the anterior trunk was observed supplying the "patellar plexus" in the suprapatellar region. In specimen two, both trunks were observed supplying the "patellar plexus" superficial to the quadriceps tendon. The MFCN was observed branching from the anterior division of the femoral nerve and traveling anteromedially superficial to the femoral artery within the triangle before

Figure 9: Lateral view of the AFCN anatomical variation in the proximal thigh from specimen two. AFCN in dark blue, MFCN in light blue, LFCN in green, and sartorius outlined in black.



piercing the deep fascia in all cases. Within the proximal thigh, the MFCN traveled medial to the sartorius before rapidly dividing into two or three main branches. These branches then quickly ascended through the adipose tissue, becoming fully subcutaneous throughout the remainder of their course **(figure 10)**. These nerves were frequently observed crossing over one another and anastomosing with other branches from the MFCN. Additionally, in all specimens, the MFCN and AFCN were observed anastomosing with each other in the mid-thigh. Ultimately, the MFCN terminated in the tissue superficial and medial to the sartorius, not supplying the "patellar plexus" in all cases.

Figure 10: Lateral view. MFCN branching throughout various subcutaneous tissue layers in the upper mid-thigh region.



The saphenous nerve was successfully located originating from the posterior division of the femoral nerve in all specimens. In specimen two, the saphenous nerve was observed traversing through the adductor canal as a single trunk before dividing into its terminal branches, the IPBSN and SBSN, just before exiting the canal. In this specimen, the IPBSN was located exiting the adductor canal along the posterior border of the sartorius demonstrating a posterior-type relationship. In specimen one, an anatomical variation was discovered in which the saphenous nerve divided into its terminal branches within the femoral triangle and traveled outside of the adductor canal medial to the sartorius. In the same specimen, the IPBSN demonstrates a posterior-type relationship to the sartorius as it crossed the muscle superficially. In all specimens, the IPBSN pierced the deep fascia and supplied branches to the skin inferior to the patella. In specimen one, three trunks of the IPBSN were observed crossing obliquely between the patella and TT. In specimen two, four total trunks of the IPBSN were located traveling obliquely, one at the level of the mid patella, two crossing at the level of the TT, and one trunk traveling below the TT. In all specimens' branches of the IPSBN were observed extending past the lateral edge of the patella. Compared to the other terminal branches (AFCN, MFCN, and LFCN), the IPBSN was adhered to the fascia for almost its whole course, only becoming subcutaneous for a short duration before terminating.

Topographic Mapping Analysis

Topographic mapping of cutaneous nerve locations supplying the "patellar plexus" revealed a general but distinct innervation pattern. In specimen one, the AFCN crossed the sartorius medial to lateral as a single trunk before dividing approximately 7.5 cm further distally into two branches **(figure 11a)**. Within the distal thigh, the lateral branch provided significantly more terminal branches and innervated the majority of the tissue superior to the patella. In specimen two, the AFCN traveled superficial to the sartorius as a single trunk and divided into its two main trunks approximately 4.69 cm distal to the muscle **(figure 11b)**. Within the distal thigh of specimen two, both branches of the AFCN were observed terminating in the suprapatellar region, with the lateral branch again providing more terminal branches.

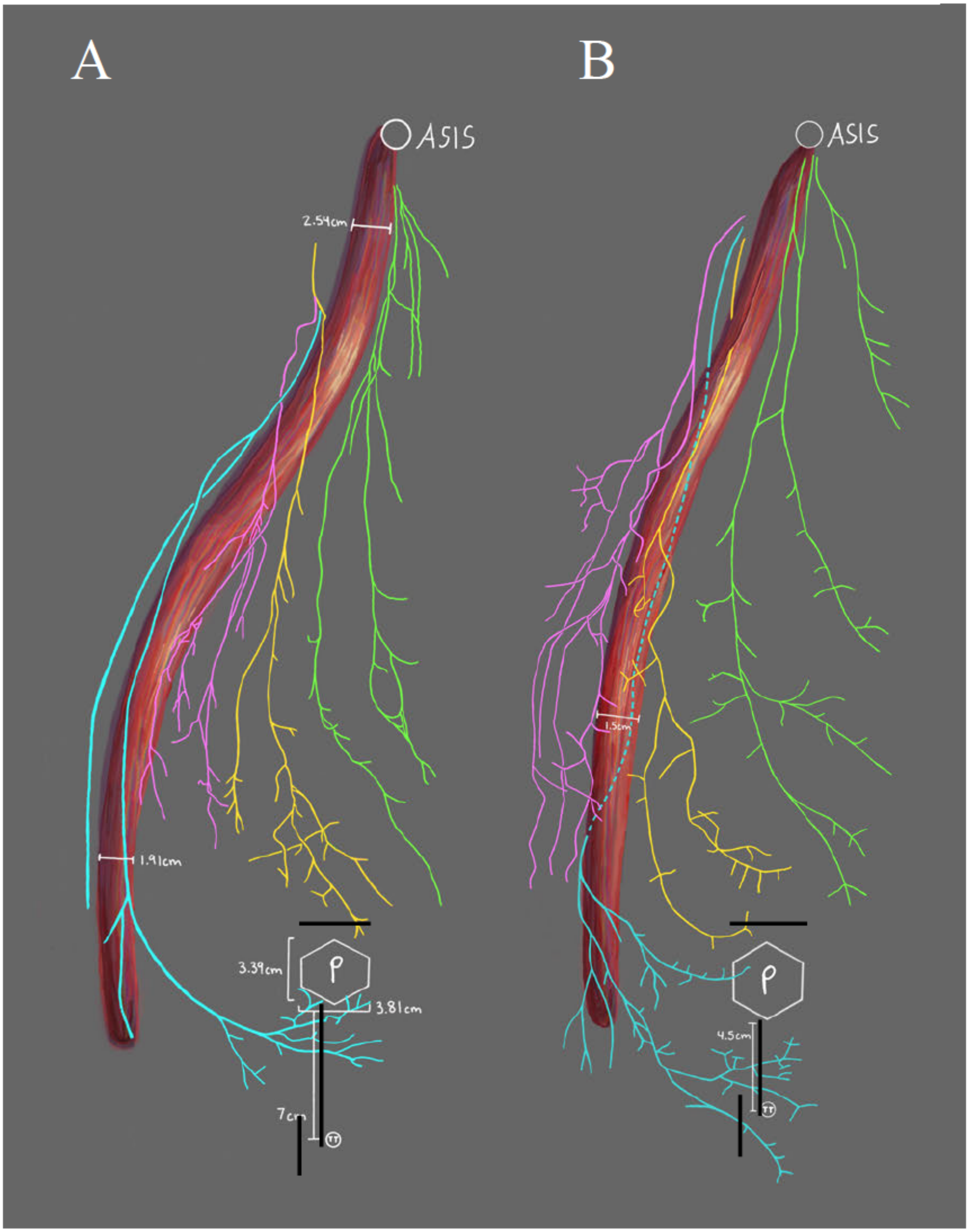
In both specimens, the MFCN also originated from the femoral nerve and exited the femoral triangle as a single trunk. In specimen one, the MFCN traveled superficial to the sartorius as a single trunk but before reaching the lateral border of the sartorius it divides into two trunks **(figure 11a)**. The posterior/medial branch rapidly divides into several branches with two of the branches crossing the sartorius and continuing distally for approximately 4.4 cm before eventually anastomosing with one another. This branch traveled 12.5 cm further distally providing several terminal branches, eventually terminating 4.3 cm lateral to the sartorius and 4.8 cm superior to the level of the superior patellar border. The anterior/lateral branch traveled as a single trunk for approximately 7.75 cm crossing the posterior branch along the lateral border of the sartorius. At this point, the MFCN divided into two dominant branches again and provided terminal branches along the sartorial border. The shorter, anterior branch traveled approximately 6.5 cm distally, terminating more than 10 cm superior to the patella, and the posterior branch traveled approximately 12.1 cm distally, terminating 6.5 cm superior to the patella. In contrast, the MFCN from specimen two did not cross the sartorius and branched entirely along the medial side of the muscle **(figure 11b)**. The main division of the MFCN was located approximately 8.17 cm distal to its origin from the femoral nerve and 0.5 cm medial to the sartorius. In specimen two, approximately 0.71 cm distal to the division, the posterior/medial branch of the MFCN further divides providing terminal branches along its approximated 12.76 cm course before terminating 6.43 cm proximal and 7.73 cm medial to the level of the superior patellar border. The anterior/lateral branch, from specimen two, continued distally as a single trunk for approximately 5.41

cm before dividing into three branches. All three branches traveled an estimated 10 cm further distally before terminating 1.68 to 2.28 cm proximal and 6.86 to 8.25 cm medial to the level of the superior patellar border.

Between both specimens, branches of the MFCN did not contribute to the “patellar plexus”. Additionally, branches of the LFCN were located within the distal thigh but swept laterally and did not contribute to the “patellar plexus”. In specimen one, the closest branch terminated approximately 3.7 cm lateral and 1.32 cm superior to the patella (**figure 11a)**. In specimen two, the LFCN terminated approximately 3.7 cm lateral and 1.32 cm superior to the patella (**figure 11b)**. In both specimens, the IPBSN provided cutaneous innervation to the infra-patellar region, extending medially from the sartorius, and crossing laterally passed the patella. In our first specimen, four branches from a single trunk of the IPBSN crossed the medial margin of the patellar ligament.

Using the measurements and landmarks for each ACLr technique, the main skin incision sites were added to the topographic map. In specimen one, the QT graft was crossed by two terminal branches of the AFCN, while zero branches of the IPBSN or SBSN crossed the HT graft. On the other hand, three trunks of the IPBSN crossed the BPTB graft **(figure 11a).** In specimen two, one terminal branch of the AFCN crossed the QT graft, one trunk of the IPBSN crossed the HT graft, and three trunks of the IPBSN crossed the BPTB graft **(figure 11b)**.

Figure 11: Topographic maps of the cutaneous innervation pattern with the main skin incisions for ACLr in black and landmarks in white (ASIS, sartorius, patella and tibial tuberosity) from specimen one A) and specimen two B). LFCN in green, AFCN in yellow, MFCN in pink, and saphenous nerve in light blue.



Ultrasound

In one cadaveric specimen, four branches of the AFCN (A-D) were identified traveling into a similar region of undissected tissue superficial to the vastus medialis muscle **(figure 13)**. Branches A, B, and D were approximated to be fully subcutaneous and approaching the region of tissue where they terminate into the subdermal plexus. Branch C was approximated to be within the deep portion of the subcutaneous tissue superficial to the investing fascia. All four branches were successfully located on a single US image plane and then confirmed on the loop images from US probe fanning. The visual approximation for all four branches was correct with branches A, B, and C all located in the superficial subcutaneous tissue and branch C located on top of the deep fascia on the US images **(figure 12).** Complete dissection and mapping of the AFCN branches confirmed our hypotheses that branches A and B were approaching their termination into the tissue. Dissection also revealed branch C was indeed a terminal branch entering the skin. Conversely, mapping of branch D revealed our hypothesis was incorrect as the nerve continued distally as a trunk before dividing further **(figure 11a).**

Figure 12: Ultrasound of the AFCN, lateral = right side of image.

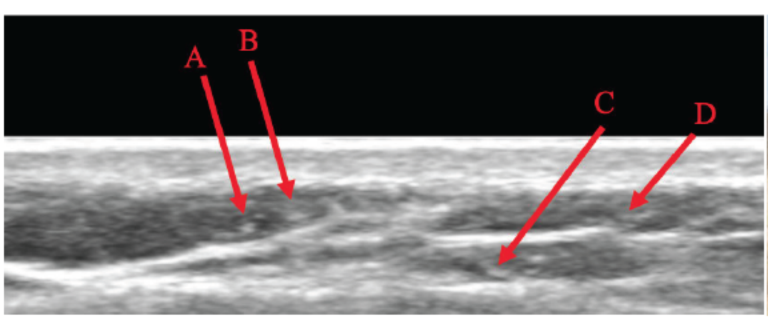
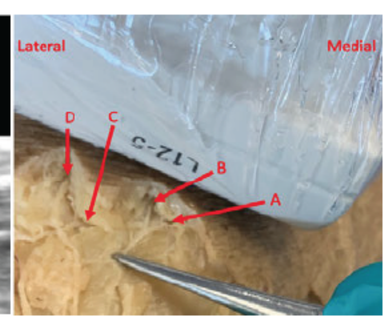


Figure 13: Branches of the AFCN with the approximate placement of the US probe used to obtained figure 14.



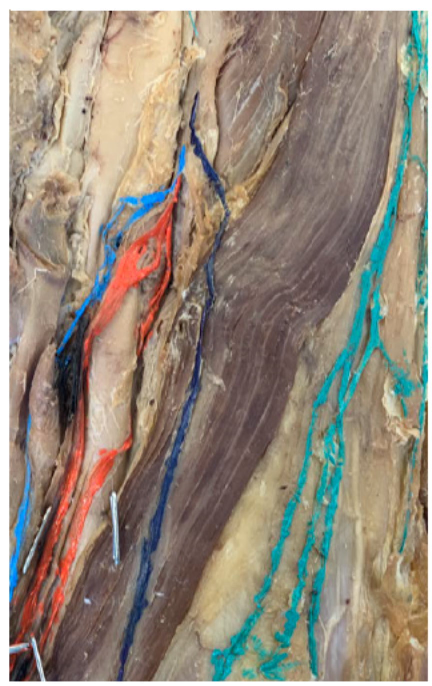
CUTANEOUS INNERVATION PATTERN OF THE ANTEROMEDIAL THIGH AND KNEE JOINT

Due to the high variability associated with the development of peripheral nerves in general, a strong anatomical foundation can aid in reducing iatrogenic damage during ACLr and subsequent sensory deficits. Knowledge of the cutaneous innervation patterns, tissue layers, nerve location, and classification (trunk or terminal branch) can assist surgeons in the placement of the skin incisions and/or where to anticipate sensory nerves during surgery.

Anterior Femoral Cutaneous Nerve

In the proximal thigh, the AFCN pierces the fascia lata and travels superficially to the sartorius. The AFCN crosses the muscle as a single trunk in the deep layer of the subcutaneous tissue, adhered to the fascia **(figure 14)**. In both specimens, the AFCN did not provide any terminal branches as this region is innervated by the femoral branch of the genitofemoral nerve. However, our recent study did identify a relatively undocumented anatomical variation in which the AFCN pierces both the sartorius and the deep fascia **(figure 9)**. As the nerve travels through the muscle, it could potentially become compressed by the muscular

Figure 14: Anterior view. AFCN in dark blue, MFCN in light blue, LFCN in green, and saphenous nerve in orange.

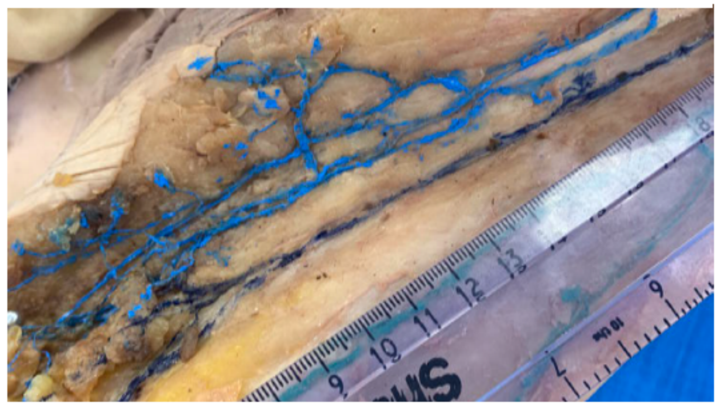


slip it creates. Although not clinically relevant for ACLr, clinicians should investigate this possibility if a patient presents with a sensory deficiency in the anterior thigh. After crossing the sartorius into the mid-thigh, the AFCN divides into its anterior and posterior branches. In this region the main branches maintain their position on the fascia and terminal branches begin to divide traveling superiorly through the adipose tissue to innervate the skin. As the AFCN travels even further distally towards the patella, the main nerve branches slowly ascend through the various subcutaneous layers, separating from the deep fascia. In the distal thigh, AFCN branches begin to deviate from the lateral border of the sartorius, taking an oblique course to the superior patellar region. Once the AFCN reaches the tissue superficial to the quadriceps tendon, the nerve consists of only small terminal branches running transversally just deep to the dermis in the superficial subcutaneous layer. At this point, the AFCN is vulnerable to damage during the QT graft skin incision.

Medial Femoral Cutaneous Nerve

Within the femoral triangle, the MFCN consists of a single trunk that generally courses medially to the femoral artery, eventually crossing superficial to the artery and piercing the deep fascia. After piercing the fascia lata, the MFCN quickly becomes fully subcutaneous, traveling in the deep subcutaneous layer, medial to the sartorius, as either a single or double trunk. Upon reaching the subcutaneous tissue superficial to the adductor longus, the MFCN rapidly divides taking a highly variable and unpredictable course **(figure 15)**. In specimen one, the MFCN crossed the sartorius laterally in this region, before branching into two dominant trunks, providing the majority of the distal terminal branches. These branches continued distally innervating the subcutaneous tissue lateral to the sartorius and superficial to the vastus lateralis. In specimen two, the MFCN rapidly divided into two trunks (medial and lateral) and several terminal branches. The medial trunk of the MFCN took an extremely superficial course through the subcutaneous tissue providing terminal branches to the adductor region. The more lateral trunk maintained a relatively deep position in the subcutaneous tissue on the medial side of the sartorius. At the mid-thigh region, the lateral trunk ascended into the superficial layer of the subcutaneous tissue continuing to branch and provide terminal branches. In contrast to the other MFCN branching pattern from specimen one, terminal branches were observed innervating tissue superficial to the adductor muscular compartment and along the medial border of the Sartorius. Lastly, as MFCN branches travel even further distally, the number and size of the terminal branches drastically diminish. In this region, the MFCN

Figure 15: Lateral view. MFCN terminal branches, in light blue, traveling with the subcutaneous tissue superficial to the adductor compartment.



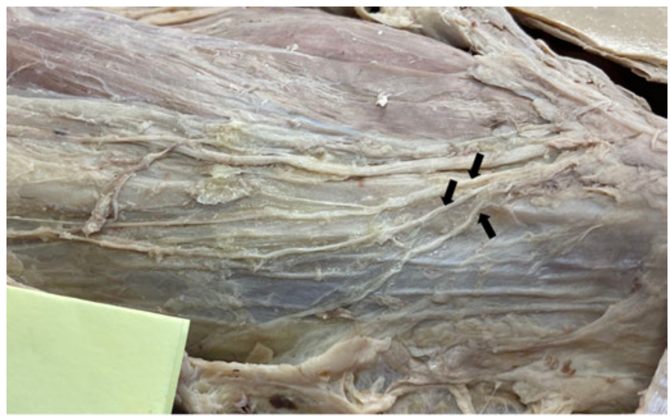
strictly consists of three to four terminal branches traveling in the superficial subcutaneous layer. These terminal branches are considerably longer than other branches and generally terminate before reaching the patella. In contrast to previous anatomical descriptions, we did not observe the MFCN contributing to the “patellar plexus” (Gray 1974).

Overall, in both specimens’ branches of the MFCN were frequently observed crossing over one another, and in some instances where terminal branches divided from the main trunk, the branches would travel together, but in different tissue planes. In both specimens, the MFCN was observed frequently anastomosing with other branches of the MFCN and AFCN. In particular, the anastomosis between the MFCN and AFCN was located in the tissue superficial to the sartorius. Additionally, during the dissection, branches of the MFCN were observed innervating superficial branches of the accessory saphenous vein and superficial femoral artery in the anteromedial thigh. The dissection results of the MFCN support the findings of Horner and Dellon's (1994) study, showing that the MFCN is the most superficial nerve terminating on the anteromedial thigh. In contrast, we did not observe any branches of the MFCN entering the adductor canal which Horner and Dellon (1994) observed in a combined 60.8% of their specimens.

Lateral Femoral Cutaneous Nerve

In both cases, the LFCN was located crossing under the inguinal ligament entering the proximal anterior thigh, medial to the ASIS but can occasionally enter laterally. In the proximal thigh, the LFCN emerges on the lateral border of the sartorius, superficial to the deep fascia. In this region, the LFCN can be classified into three types based on the branching pattern and dominant branches. During our dissection, we observed one fan-type and one sartorius-type LFCN branching pattern. Within the proximal thigh, the sartorius-type pattern, from specimen two, consisted of two dominant anterior trunks tightly adhered to the deep fascia on the lateral border of the sartorius. These branches travel distally into the mid-thigh along the sartorius providing minimal terminal branches. The smaller trunk deviates from the sartorius first, sweeping laterally across the thigh, terminating into the subcutaneous tissue superficial to the rectus femoris and vastus lateralis. The remaining, more dominant trunk continues into the distal thigh before sweeping laterally and becoming fully subcutaneous. In our specimen, the nerve then divided into two branches continuing laterally before terminating over the distal vastus lateralis. In the fan-type branching pattern from specimen one, two trunks of the LFCN were also identified entering the proximal superficial to the deep fascia. Within the proximal thigh, the posterior branch quickly divided into three equal-sized branches traveling posteriorly **(figure 16).** The more dominant anterior branch then took a similar course to the sartorius-type pattern, traveling and branching along the lateral border of the sartorius. Again, in the mid-thigh,

Figure 16: Lateral view. Posterior branches of the LFCN from specimen one, demonstrating a fan-type branching pattern.



the LFCN separates from the deep fascia, deviating from the sartorius and sweeping laterally. Once in the distal thigh, the LFCN branches were fully subcutaneous, consisting of mainly terminal branches ending in the subcutaneous tissue superficial to the rectus femoris and vastus lateralis. In contrast to other anatomical descriptions, we did not observe any branches of the LFCN supplying the "patellar plexus" (Gray 1974; Kampitak et al. 2021; Surucu et al. 1997).

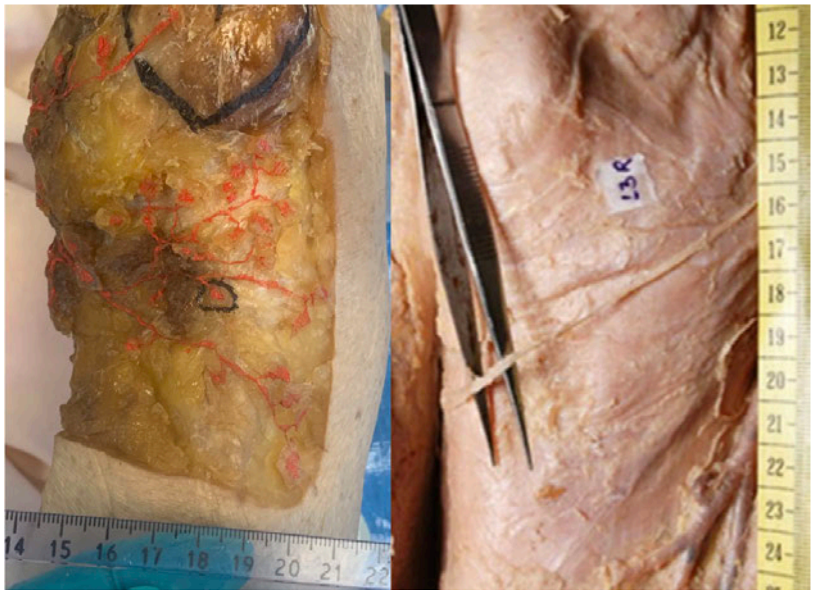
Saphenous Nerve

In specimen two, the saphenous nerve demonstrated its typical branching pattern originating from the posterior division of the femoral nerve as a single trunk within the femoral triangle. After branching from the femoral nerve, the saphenous nerve travels distally deep to the sartorius and fascia lata with the superficial femoral artery into the adductor canal. Within the distal portion of the adductor canal, the saphenous nerve pierces the vasto-adductor membrane and deep fascia exiting the canal and eventually dividing into the IPBSN and SBSN. Of the 45 specimens dissected by Horner and Dellon (1994), they observed the IPBSN dividing from the main saphenous nerve in this location, the distal/inferior thigh, in 24% of their specimens. At this point, the IPBSN typically emerges superficially along the posterior/medial border of the sartorius and crosses anteriorly over the muscle towards the infra-patellar region. In specimen two, an anatomical variation was discovered in which after originating from the femoral nerve, the saphenous nerve quickly divided into two trunks. These trunks, later confirmed as the IPBSN and SBSN, followed a similar course traveling distally with the superficial femoral artery. Horner and Dellon (1994) also observed this branching pattern, reporting in 18% of the specimens the IPBSN divided in the proximal thigh.

Additionally, in specimen two, after reaching the entrance of the adductor canal, the saphenous nerve trunks traveled superficial to the aponeurosis of the vastus medialis, which forms the roof of the adductor canal, never entering the canal. The IPBSN and SBSN continued distally, running in parallel along the posterior/medial border of the sartorius. In the mid-thigh, the IPBSN deviated from the SBSN crossing the sartorius superficially and continuing into the distal thigh along the anterior/lateral border of the sartorius. At the level of the patella, the IPBSN divides into two main branches which swept laterally to innervate the infra-patellar region. In Arthornthurasook's (1988) study of 20 cadavers, they found the posterior relationship was the most prevalent, occurring in 62% of the limbs studied. They also observed that in 22% of the limbs the IPBSN pierced the sartorius, and in 3% of the limbs, the IPBSN emerged along the anterior border of the sartorius (Trescot, Brown, and Karl 2013). Finally, in 14% of the limbs, Arthornthurasook (1988) observed the IPBSN traveling in parallel and posterior to the sartorius, similar to the anatomical variation we identified. Unfortunately, there was no mention of the relationship of IPBSN and the adductor canal in this branching pattern. In contrast to the results of Arthornthurasook's (1988) study, Kalthur et al. (2014) dissected 32 lower extremities from 16 donors, finding the IPBSN emerged from the anterior border in 68.7% of the specimens, the IPBSN pierced the muscle in 28.1% of the specimens and in only 3.1% of the specimens the IPBSN emerged from the posterior border of the sartorius. The course of the IPBSN after exiting the adductor canal is extremely variable and has been reported to vary between an individual's left and right leg (Kalthur et al. 2014). In most individuals, Horner and Dellon (1994) reported that two or more branches exit the adductor canal proximal to the knee joint, with the anterior branch being the IPBSN and the more posterior branch being the SBSN (Trescot, Brown, and Karl 2013). The dissection results from both of our specimens confirmed this classification. In specimen two, with a more typical branching pattern, three trunks emerged from the posterior border of the sartorius. The two more anterior/superior branches were confirmed as branches of the IPBSN, and the more posterior branch was confirmed as the SBNS. This classification was also true for specimen one, in which the saphenous nerve did not enter the adductor canal. Proximal to the knee joint the more anterior branch represented the IPBSN and the posterior branch represented the SBSN.

After branching from the main saphenous nerve, Mochida and Kikuchi (1995) identified two predominant branching patterns for the IPBSN. They observed in 68% of the cadavers, the IPBSN crossed the knee joint medially, at the level of the patellar ligament and in 32% of the cadavers the IPBSN crossed the patellar ligament proximal/superior to the knee joint (Trescot, Brown, and Karl 2013). Additionally, Kalthur et al. (2014), observed one "terminal branches" in 40.6% of the donors, two "terminal branches" in 34.3% of the donors, and three "terminal branches" in 25% of the donors. Comparing our more detailed dissection results to images from Kalthur et al. (2014), it is apparent that Kalthur et al. (2014) observed one to three trunks adhered to the deep fascia rather than "terminal branches" which should be traveling through the subcutaneous layers **(figure 17)**. During our dissection, we were able to preserve the actual terminal branches as they divided from the main IPBSN trunk(s) and follow them until they terminated into the tissue inferior to the patella. Nevertheless, of the 13 donors demonstrating a single trunk of the IPBSN, Kalthur et al. (2014) reported 11 of the trunks passed between the patella and TT, one trunk passed at the level of the patellar apex and one trunk passing inferior to the TT. In the 11 donors with two trunks of the IPBSN, Kalthur et al. (2014) observed two trunks traveling between the patella and TT in four donors and in three donors the trunks were located at the level of the TT. In one donor, Kalthur et al. (2014) observed one trunk traveling at the level of the patella with the second traveling between the patella and TT. Kalthur et al. (2014) also reported in two donors, one trunk traveling at the level of the patella with the other trunk traveling between the patella and TT. In the last donor, one trunk was observed traveling between the patella and TT with the second trunk at the level of the TT. Lastly, within eight out of 32 donors, three

Figure 17: Comparison of IPBSN dissection results, right image taken from Kalthur et al. (2014), and left image obtained from our study.



branches of the IPBSN were observed four main patterns were found. In two donors Kalthur et al. (2014) observed all three branches traveling between the patella and TT and in one donor all three branches traveled at the level of the TT. Additionally, in one donor, a single branch was observed at the patella with the remaining two branches traveling between the patella and TT, two donors had one branch between the patella and TT with the remained traveling to the TT and in the final donor, two branches were located between the patella and TT with only a single branch at the level of the TT (Kalthur et al. 2014). During our study, in specimen one, we identified two trunks of the IPBSN and in specimen two we identified three trunks. In the specimen with two trunks, the superior trunk was located traveling between the patella and TT, and the inferior trunk terminated before reaching the infra-patellar region. The superior trunk then divided into three branches innervating the tissue in this region. In our other specimen, a single superior trunk was observed traveling to the midpoint of the patella before terminating along its medial margin. The remaining two inferior trunks were located at the level of the TT with the more dominant trunk crossing superiorly to the TT and the smaller crossing inferiorly.

SURGICAL IMPLICATIONS

In conjunction with the current literature, our study has confirmed that the femoral cutaneous and saphenous nerves are highly variable from individual to individual and can vary between the left and right lower extremities (Kalthur et al. 2014). With 13% to 76.3% of patients reporting sensory deficits following ACLr using a HT graft, 40% to 77% following a BPTB graft, and 48% to 86% following a QT graft, surgeons need to utilize a combination of anatomical knowledge and pre-operative techniques to reduce iatrogenic nerve damage (Sabat and Kumar 2013; Ruffilli et al. 2016; Kartus, Movin, and Karlsson 2001; Dai et al. 2021; Haviv et al. 2017; Hopton, Tommichan, and Howell 2004; Lund et al. 2014). As demonstrated in our dissection study, the anatomy of the femoral cutaneous and saphenous nerve branches, apart from the branching pattern, is generally unique in each region of the thigh/knee. For example, in the mid-thigh, the AFCN tightly adheres to the deep fascia providing minimal terminal branches but in the same region, the MFCN is almost fully subcutaneous and rapidly dividing into terminal branches. The results of our topographic mapping can be used by surgeons to compare similar branching patterns and identify the location(s) of the main trunk(s). Iatrogenic damage to a single terminal branch results in minimal sensory deficits compared to damaging a main trunk, which would result in a significantly larger area of sensory deficits distal to the point of injury. In addition, our combined dissection and mapping results can be used to predict the location of these main trunks and guide the skin incision to avoid the nerve branches. This has already been implemented during ACLr using a HT graft to avoid the IPBSN. In the systemic review conducted by Ruffilli et al. (2016), the rate of post-operative sensory loss using a vertical incision was between 33% and 65.7% compared to an oblique incision which was between 13% and 24%. Based on the literature and our results it is clear that an oblique incision minimizes nerve damage as it mimics the anatomical course of the IPBSN in this region. Overall, relying solely on anatomical knowledge could potentially reduce sensory deficits following ACLr but with detailed descriptions limited to our two specimens, more studies are required to validate our findings.

But even with strong anatomical knowledge and adopting an oblique skin incision, sensory deficits remained in some patients following HT graft ACLr. Because of the high variability demonstrated by the IPBSN using standardized distances from anatomical landmarks or “safe zones” for the skin incision placement is inadequate. In theory, two identical individuals could receive an oblique incision in the same location but have different sensory deficits following surgery. The development of a rapid and accurate pre-operative procedure to locate the nerve branches prior to the skin incision would allow surgeons to account for the variability of the sensory nerves between individuals. Hu et al. (2015) proposed a method of localizing the cutaneous nerves of the patella region in 25 healthy volunteers using a transcutaneous electrical stimulation (TENS) wand. To locate the sensory nerves inferior to the patella, the TENS wand was traced over the skin and the areas of maximal TENS response were located using subject feedback and marked (Hu et al., 2015). These locations were retracted with the TENS wand at incrementally lower currents until pain or sensation in the region was no longer reported by the subject and the final nerve location was marked (Hu et al., 2015). The major disadvantages of TENS are the time required to trace the skin, the pain associated with electrical stimulation, and the skill of the clinician operating the device.

Because of the enormous applications and versatility of US imaging in modern healthcare, point-of-care ultrasound (POCUS) has become standard clinical practice, with many medical schools integrating US training into their curriculum (Landau-Taylor et al., 2022). Diagnostic US is relatively simple to operate, cost-effective, non-invasive, and allows clinicians to rapidly acquire high-resolution images at the bedside. Sensory nerves in the transverse plane were identified as honeycomb-like hypoechoic nerve fascicles surrounded by a hyperechoic interfascicular connective tissue (Yeo et al., 2020). During our study, we successfully demonstrated the ability to locate terminal branches of the AFCN in the superior patellar region on US as tiny echogenic foci. Additionally, through dissection and topographic mapping, we were able to confirm the structures identified on US as branches of the AFCN. Traditional pre-operative protocols for ACLr use standardized anatomical landmarks and distances for the placement of the skin incisions. These protocols do not account for the anatomical differences between individuals of different biological sexes, heights, BMI, and the high variability in sensory nerve location. Development of a standardized pre-operative US protocol would provide clinicians with a reliable, accurate, and fast method of locating sensory nerves before the first incision is made. In combination with current pre-operative protocols, US imaging would allow surgeons to scan the region of skin and place the proposed incision site in a location with minimal to no sensory nerves for each patient. Although we demonstrated the feasibility of locating terminal branches of the AFCN on US, more research is required to determine the optimal transducer type, software, imaging mode, and settings to consistently identify the more distal nerve branches. To enhance future cadaveric US studies, it may be beneficial to incorporate techniques such as applying light traction to the more proximal dissected part of the nerve and observing the movement of the echogenic foci on the US images, providing further confirmation of the nerve's location. More studies are also needed to explore locating the IPBSN on US, as it is frequently damaged during ACLr. Lastly, a comprehensive US study of the cutaneous nerves at the knee joint involving human participants would provide the necessary results to implement a standardized pre-operative US protocol for ACLr.

CONCLUSION

Based on our results and current literature, the cutaneous innervation of the knee joint is extremely variable and difficult to predict. Using only traditional anatomical landmarks and distances for skin incisions is insufficient in reducing iatrogenic nerve damage. However, we have demonstrated that terminal branches can be located on US, and with further research, a potential pre-operative protocol could be developed. Unfortunately, due to the meticulous dissection performed, only two embalmed cadaveric specimens were analyzed in our study. As a result, our focused shifted from producing spatial heat maps which would help identify potential “safe zones” for surgical innervation to topographic maps allowing us to further define the subcutaneous branching patterns of the sensory nerves. This mythology did allow us to maintain the location of each nerve branch and define the anatomical characteristics in different regions of the thigh and knee joint. Additionally, based on the specimens available we were limited to only biological females and did not have the opportunity to compare left and right lower extremities. In the most comprehensive anatomic study of the IPBSN to date, Kalthur et al. (2014) only investigated the branching pattern in biological male specimens, despite biological females having a significantly higher incidence of ACL injuries. Future research should include both biological sexes to potentially identify common branching patterns within the same sex and/or between sexes. Lastly, the measurements from the topographic mapping were obtained using an open-access photo tool, which uses a known distance in a photo as a reference point. Because this tool functions in a two-dimensional plane it is difficult to accurately measure the three-dimensional shape of the nerves as they travel distally down the thigh. Additionally, in a two-dimensional plane measurement of nerves branching into different subcutaneous tissue layers is inherently less accurate than using a digital caliper. Ultimately, the results of our study have provided the framework needed for future dissection and US research. Ideally, these studies should include a large sample size and both biological sexes to ensure the results represent the true population and be implemented into ACLr.

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