



## Original Article

# Histological study of the posterior cruciate ligament femoral insertion<sup>☆</sup>



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## ABSTRACT

**Objectives:** To describe the microscopic anatomy of the posterior cruciate ligament femoral insertion in order to identify and establish differences between the direct and indirect insertions of this ligament.

**Methods:** Ten cadaveric knees were used for this study. The posterior cruciate ligament femoral insertion was observed microscopically. Hematoxylin and eosin staining was performed to observe the morphology of the posterior cruciate ligament insertion. Alcian blue staining was performed to determine the location of the cartilage matrix and better assist in the observation and differentiation between direct and indirect insertions.

**Results:** The direct insertion was observed to be a more complex structure than the indirect insertion because it showed four different histological layers (ligament, uncalcified fibrocartilage, calcified fibrocartilage, and bone). Chondrocytes were observed in the uncalcified and calcified fibrocartilage layers. It was observed that the indirect insertion was composed of two layers in which the ligament was anchored directly to the bone by collagen fibers. Indirect insertion was located in the marginal region of the posterior cruciate ligament between the direct insertion and the anterior articular cartilage.

**Conclusion:** Through histological analysis, it was demonstrated that the indirect insertion was adjacent to the anterior articular cartilage and presents a histological pattern where the collagen fibers insert directly into the bone (two-layer insertion). The direct insertion is posterior to the indirect insertion and has four histologically distinct layers.

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## Estudo histológico da inserção femoral do ligamento cruzado posterior

### R E S U M O

#### Palavras-chave:

Ligamento cruzado posterior/anatomia & histologia  
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Cadáver

**Objetivos:** Descrever a anatomia microscópica da inserção femoral do ligamento cruzado posterior a fim de identificar e estabelecer diferenças entre as inserções direta e indireta desse ligamento.

**Métodos:** Foram usados dez joelhos procedentes de amputações transfemorais. A inserção femoral do ligamento cruzado posterior foi observada microscopicamente. A coloração hematoxilina e eosina foi feita para observar a morfologia da inserção do ligamento cruzado posterior. A coloração azul de Alcian foi feita para determinar a localização da matriz de cartilagem e melhor ajudar na observação e diferenciação entre a inserção direta e indireta. **Resultados:** Observou-se que a inserção direta do ligamento cruzado posterior é uma estrutura mais complexa do que a inserção indireta, por apresentar quatro camadas histológicas distintas (ligamento, fibrocartilagem não calcificada, fibrocartilagem calcificada e osso). Os condrócitos foram observados nas camadas não calcificadas e calcificadas de fibrocartilagem. Foi observado que a inserção indireta, composta de duas camadas nas quais o ligamento está inserido diretamente ao osso por fibras de colágeno, está localizada na região marginal do ligamento cruzado posterior entre a inserção direta e a borda da cartilagem anterior do côndilo.

**Conclusão:** Através de análise histológica, o presente estudo demonstrou que a inserção indireta do ligamento cruzado posterior situa-se adjacente à borda da cartilagem anterior do côndilo femoral e apresenta um padrão histológico no qual as fibras de colágeno se inserem diretamente no osso. A inserção direta encontra-se posterior à inserção indireta e apresenta quatro camadas histológicas distintas.

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## Introduction

The posterior cruciate ligament (PCL) is considered the primary stabilizer of posterior translation of the tibia and a secondary stabilizer of varus, valgus, and external rotation of the knee. The PCL is composed of two main bundles, the anterolateral (AL) and the posteromedial (PM).<sup>1</sup> The two bundles increase their length when the knee is flexed, suggesting a greater functionality in flexion than in extension.<sup>2</sup> Clinical and biomechanical studies in which the two bands were reconstructed presented superior results than those that adopted single-band reconstruction.<sup>3,4</sup> This technique relies on a precise identification of the PCL's femoral insertion points, so that the grafts can correctly resist the posterior translation of the tibia in relation to the femur through varying degrees of knee flexion.<sup>5</sup> Tunnel positioning, particularly in the femur, is currently considered to be one of the most important factors in the reestablishment of the kinematics of this joint and, consequently, in the clinical results of anatomical reconstruction of the PCL.<sup>6,7</sup>

From a microscopic standpoint, the insertion of the ligament to the bone has two distinct structures: direct and indirect insertion.<sup>8-11</sup> These two types of insertion are histologically different. While direct insertion has four histological layers (ligament, non-calcified fibrocartilage, calcified fibrocartilage, and bone), the indirect insertion presents only two (the ligament is inserted directly into the bone, without a clear transition between them). Recent studies involving

microscopic analysis of the anterior cruciate ligament (ACL) have assessed the histological and functional differences between these insertions.<sup>8-10</sup> Regarding PCL, the literature presents conflicting information on the correct position of its femoral insertion; many authors have described different methods of locating the anatomical site of femoral insertion, but few have demonstrated its microscopic anatomy.<sup>5,12-14</sup>

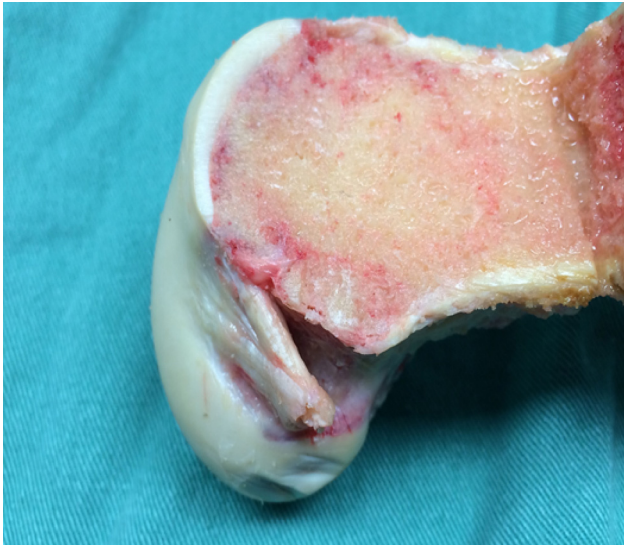
More precise anatomical data may help surgeons to choose the best location for the femoral tunnel during the reconstruction of this ligament, as a clearer understanding of the macroscopic and microscopic anatomy of the femoral insertion site can minimize femoral tunnel positioning errors.

The present study is aimed at describing the microscopic anatomy of the femoral insertion of the PCL in order to identify and determine the differences between the direct and indirect insertions of this ligament.

## Methods

This study was approved by the research ethics committee of this institution (CAAE: 32641114.0.0000.5479).

Ten knees (five males and five females) from transfemoral amputations due to vascular problems were used. Exclusion criteria included any signs of prior surgery, ligament damage, and signs of moderate/severe osteoarthritis, which would hinder data analysis. Ligament injury and osteoarthrosis were subjectively assessed by two surgeons at the time of dissection, who reached a consensus regarding the inclusion or

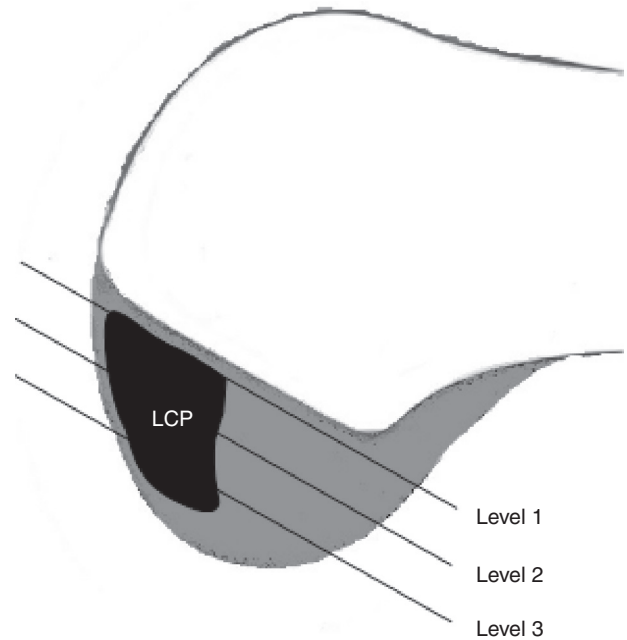


**Fig. 1 – Macroscopic appearance of the medial femoral condyle with the PCL insertion area.**

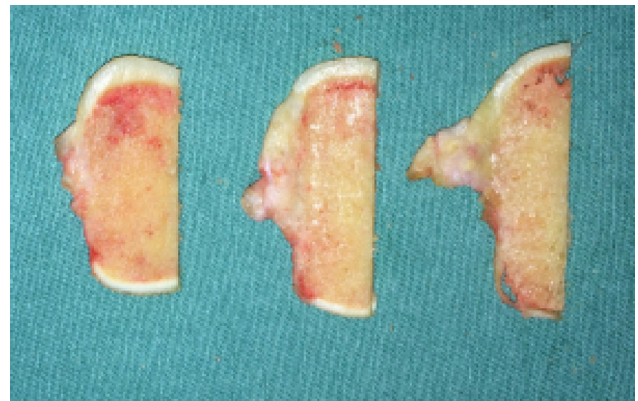
exclusion of the knee in the study. Prior to dissection, the knees were stored in 10% formaldehyde and kept refrigerated at 5.3 °C. The mean age of the samples was 61.2 years (42–77). The dissections were performed at the institution's morgue. For dissection of the anatomic planes, a longitudinal incision was made at the midline of the knee and extended 5 cm above the upper pole of the patella toward the anterior tibial tuberosity. The soft tissue structures around the knee were removed to expose the joint. Then, using an oscillating saw, the lateral femoral condyle was osteotomized using the inverted-L technique for complete visualization of the PCL insertion into the medial femoral condyle (Fig. 1). The PCL body was sectioned, while the proximal stump of the ligament was kept inserted into the femoral condyle to allow visualization of the ligament insertion into the bone and to assess the morphology and femoral position of the ligament in the medial femoral condyle area. Subsequently, the medial condyle was cut with a circular saw in an axial oblique orientation, parallel to the intercondylar roof, in three levels (Fig. 2); therefore, at the end of the dissections there were ten samples for level 1, ten for level 2, and ten for level 3, totaling 30 samples (Fig. 3).

### Microscopic evaluation

After macroscopic observation, each sample was fixated in a formalin solution (10% buffer-neutral) for two days. Decalcification was done in ethylene diamine tetraacetic acid (EDTA) for two to four days, depending on the bone quality. The samples were dehydrated in a series of graduated alcohols. Thereafter, the blocks (samples) were soaked in paraffin wax and cut into 5- $\mu$ m thick slices, in the same direction in which they had been cut during dissection. The adjacent sections were then stained with hematoxylin and eosin (H&E) and Alcian blue at pH 2.5. H&E staining was performed to analyze the morphology of the PCL insertion. Alcian blue staining was performed to determine the location of the cartilage



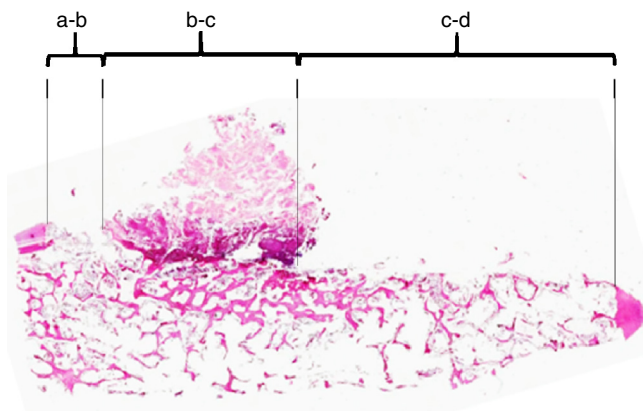
**Fig. 2 – Femoral condyle cut levels. The samples were cut in an oblique axial plane, parallel to the intercondylar roof.**



**Fig. 3 – Macroscopic appearance of the three levels at which the medial femoral condyle was cut.**

matrix and better assist in the observation and differentiation between direct and indirect insertions.

To quantify the area of insertion, its sections were carefully observed with a standard light microscope (Leica GalenIII), without the use of polarized lens, at 10 $\times$ , 20 $\times$ , and 40 $\times$  magnification. Each measurement was performed at three different levels, as there were three samples for each knee. The following measurements were taken: distance from the anterior to the posterior cartilage border of the femur (a–d); distance from the anterior cartilage border to the anterior margin of the PCL (a–b); distance from the posterior margin of the PCL to the posterior cartilage border of the medial condyle (c–d); and distance from the anterior to the posterior margin of the PCL (b–c; Fig. 4). The measurements were calculated using the optical system composed of three lens systems (ocular, objective, and condenser) and a light source, to which a micro- and millimeter ruler was coupled. When the image



**Fig. 4 – Microscopic measurements: (a-b), distance from the anterior cartilage border to the anterior margin of the PCL; (b-c), distance from the anterior to the posterior margin of the PCL; (c-d) distance from the posterior margin of the PCL to the posterior cartilage border of the medial condyle; and (a-d) sum of all the measurements, representing the distance from the anterior to the posterior cartilage border.**

was enlarged, it was possible to establish the areas of interest and to obtain the necessary measures with greater precision (Fig. 4). The measurements were presented as means and standard deviations for each level (1, 2, and 3) and for the total of the analyzed specimens.

## Results

### Macroscopic evaluation

In all specimens, the femoral insertion of the PCL took the shape of one-quarter of an ellipse, varying only in size. The anterior margin of the PCL was adjacent to the medial femoral condyle cartilage, and a free space between the margin of the ligament and the border of cartilage was not observed. The ligament insertion was located almost entirely in the anterior half of the femoral condyle.

### Microscopic evaluation

Four layers were observed in the central region of the PCL insertion (direct insertion) with H&E staining: the ligament itself, non-calcified fibrocartilage, calcified fibrocartilage, and bone (Fig. 5A). The direct insertion of the PCL was observed to be a more complex structure than the indirect insertion, since it contained these four distinct histological layers. No similar structures were observed around the PCL insertion. Chondrocytes were observed in the non-calcified and calcified layers of fibrocartilage. It was observed that the indirect insertion, composed of two layers in which the ligament is inserted directly to the bone by collagen fibers, is located on the border region of the PCL, between the direct insertion and the border of the anterior cartilage of the condyle (Fig. 5B). With Alcian blue staining, the indirect insertion area was not stained and the uncalcified and calcified layers of cartilage were positioned a few millimeters away from the border of the anterior cartilage

**Table 1 – Microscopic measurements of the posterior cruciate ligament (PCL) femoral insertion.**

Level	a-d	a-b	b-c	c-d
1	30.2 ± 3.9	3.9 ± 2.0	13.2 ± 9.9	13.1 ± 2.6
2	26.2 ± 4.4	3.1 ± 1.9	13.4 ± 2.5	10.2 ± 0.2
3	23.8 ± 2.6	3.4 ± 0.6	12.5 ± 2.1	7.9 ± 3.6
Total	26.9 ± 1.4	3.4 ± 1.6	13.1 ± 2.1	10.4 ± 3.5

Data expressed as mean ± standard deviation (in millimeters).

a-d, distance from the anterior to the posterior cartilage border of the femoral condyle; a-b, distance from the anterior cartilage border to the anterior margin of the PCL; b-c, distance from the anterior to the posterior margin of the PCL; c-d, distance from the posterior margin of the PCL to the posterior cartilage border of the medial condyle.

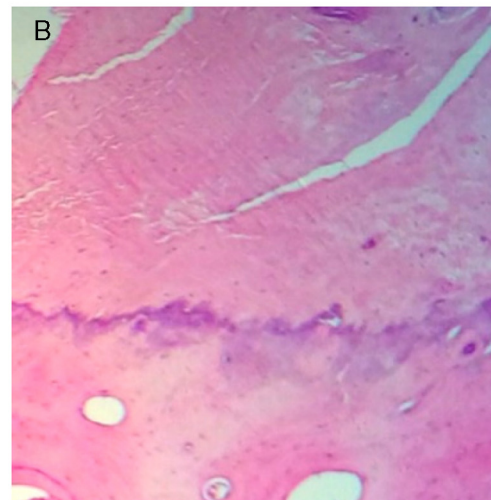
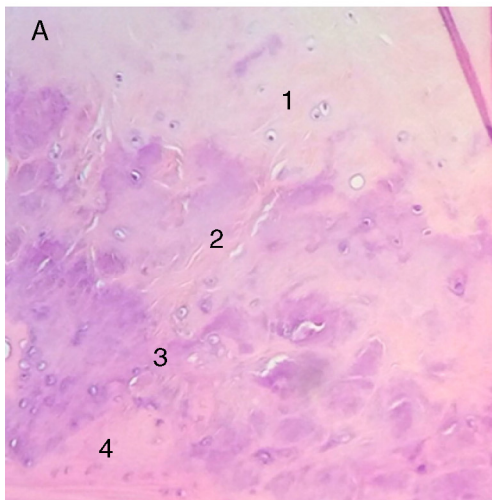
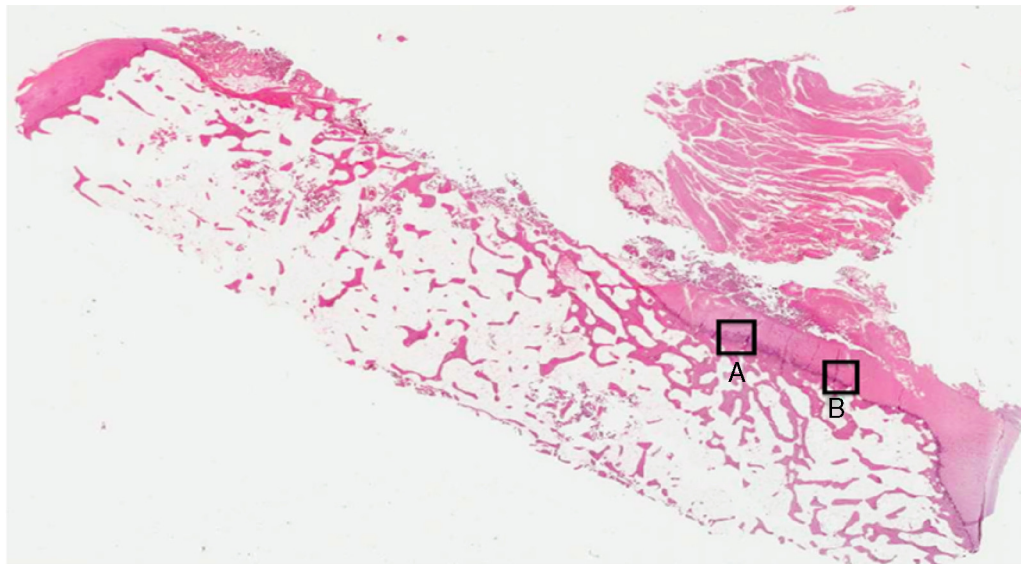
(Fig. 6). Table 1 presents the microscopic measurements of the PCL femoral insertion.

## Discussion

The most important finding of the present study was the histological analysis, which demonstrated that the femoral insertion of the PCL is composed of two different structures: a direct and an indirect insertion. The indirect insertion was marginal to the direct insertion and adjacent to the anterior cartilage border of the medial femoral condyle.

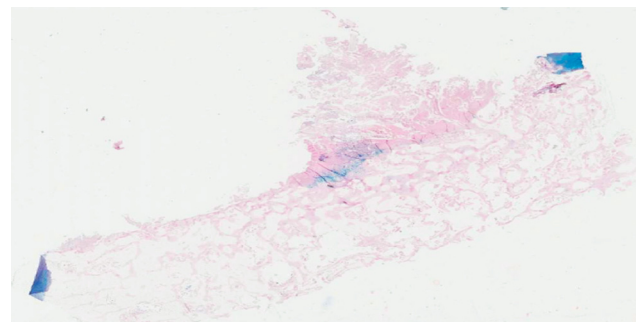
Subit et al.<sup>11</sup> described the microstructure of the femoral and tibial insertion of the PCL into the bone; however, those authors did not determine the differences between direct and indirect insertion. They used optical microscopy to demonstrate that ligament insertion to the bone has a transition zone composed of noncalcified fibrocartilage and calcified fibrocartilage, concluding that the PCL bone insertion has a histological structure similar to the other ligaments and tendons previously described in the literature. In the present study, it was possible to observe and determine the histological differences between the direct and indirect insertion of PCL femoral insertion, similar to that described by Sasaki et al.<sup>8</sup> in the histological analysis of the ACL femoral insertion. It was observed that the direct insertion is composed of four histological layers (ligament, non-calcified fibrocartilage, calcified fibrocartilage, and bone), whereas in the indirect insertion the ligament is inserted directly into the bone by collagen fibers and the four layers described for direct insertion are not distinguishable. It was not possible to establish a distinction between direct and indirect insertions on macroscopic observation.

The differentiation between the two types of ligament insertions into the bone (direct and indirect) has been addressed by several other recent studies.<sup>8-10,15</sup> Studies that assessed the ACL demonstrated that the direct insertion, located predominantly in the most central portion of the ligament, played an important role in the mechanical attachment of the ligament to the bone, in contrast to the indirect insertion.<sup>15,16</sup> Uncalcified fibrocartilage, present only between the structural layers of the direct insertion, plays an important role in controlling the tension that occurs over the ligament, compatible with that observed in muscle contraction.



**Fig. 5 – PCL femoral insertion histology observed with H&E staining. (A) Direct insertion, composed of four layers: (1) ligament, (2) noncalcified fibrocartilage, (3) calcified fibrocartilage, and (4) bone (10× magnification). (B) Indirect insertion composed of two layers: ligament and bone.**

<sup>17</sup> Considering that indirect insertion acts as a dynamic anchor of the soft tissues to the bone, allowing certain shear movements, the anchoring force is weaker than in direct insertion.<sup>15-18</sup> Moulton et al.<sup>9</sup> demonstrated that the direct insertion fibers were more deeply inserted into the bone and had more interdigitations when compared with those of the indirect insertion. These findings suggest that the ideal location for the femoral tunnel is in the direct insertion of the PCL. In addition to the histological analysis of the femoral insertion of the PCL, measurements of its insertion were obtained in order to evaluate the relationship between the ligament insertion with the anterior cartilage border of the femoral condyle. Similar measurements were made by Sasaki et al.<sup>8</sup> and Iwahashi et al.,<sup>10</sup> but those authors assessed the femoral insertion of the ACL. The present authors believe that the analysis of these measurements can help the surgeon in achieving a correct location of the femoral tunnel (or tunnels, depending on the technique used) in the surgical reconstruction of the PCL,



**Fig. 6 – PCL femoral insertion histology observed with Alcian blue staining. Calcified fibrocartilage is stained, thus highlighting the direct insertion of the ligament.**

since the ideal would be to position the tunnel in the central portion of the ligament, where the fibers of the direct insertion are located.

The present study has as limitations the low number of samples used for the analyses, which may not have accounted for the complete spectrum anatomical variation of this ligament. The study also evaluated the PCL as a whole, rather than assessing the bands separately, which is a limitation for the use of this data in double-band PCL reconstruction. Individual differences such as gender and knee size were observed, which may influence the positioning and size of the ligament insertion. In addition, the mean age of 61.2 years may also have affected the measurements, due to an already existing ligament degeneration. The measurements found in this study are aimed at assisting in the location of the femoral insertion of the ligament, but the surgeon should take into account individual differences at the time of PCL surgical reconstruction.

## Conclusion

Through histological analysis, it was demonstrated that the femoral insertion of the PCL is composed of two structures: a direct and an indirect insertion. The indirect insertion is adjacent to the anterior cartilage border of the femoral condyle and presents a histological pattern in which the collagen fibers are inserted directly into the bone. In turn, the direct insertion is posterior to the indirect insertion and presents a histological pattern with four distinct layers: ligament, non-calcified fibrocartilage, calcified fibrocartilage, and bone.

## Conflicts of interest

The authors declare no conflicts of interest.

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