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Histological analysis of the anterolateral ligament of the knee

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ABSTRACT

Anterolateral ligament (ALL) was recently described as an important structure to control the pivot-shift phenomenon in the knee. Doubts remain regarding its origin and histological properties. The purpose of this study was to identify the ALL histological structure comparing its characteristics with those of the anterior cruciate ligament (ACL) and joint capsule. ALL was harvested in 25 knees during a total knee arthroplasty (TKA) and histologically evaluated investigating for orientation of fibers, adipose tissue, presence of proprioceptors and synovial like coating. Analysis showed significant differences in several aspects between capsule and ALL; analogies were found comparing the ALL with ACL.

1. Introduction

A complex system of static and dynamic structures makes the knee stable to translational and rotational forces; in fact, this joint is a complex apparatus constituted by several intra-articular and extra articular stabilizing structures able to contrast and balance cyclic anteroposterior and rotatory forces.[1] Despite many studies conducted to fully understand the anatomy of the knee, the relationship between several anatomical structures in the lateral compartment of the native knee and their functions have not been completely understood, especially during the active motion.[2,3]

In the recent literature, the anterolateral ligament (ALL) has been subject to debate: described by numerous authors, this structure connects the lateral femoral condyle with the lateral meniscus and the proximal tibia in the lateral compartment of the knee.[4] Unfortunately, several characteristics of the ALL are still not fully understood causing uncertain: its anatomical description, the histological constitution and its biomechanical properties are still unclear.

The ALL has been described for the first time in the 1879 by Segond[5]: in this study, the ALL was described as a "pearly, resistant and fibrous band" reinforcing the joint capsule on the lateral side of the knee limiting the excessive internal tibial rotation. In subsequent years, several definitions of the ALL followed: it was reported as "lateral short ligament" by Last in 1948,[6] as a "lateral capsular ligament" by Hugston et al., in 1976[7] or as an "anterior oblique band" by Campos et al. more recently[8]; finally, it has been identified with the term of anterolateral ligament by Vieira et al.[9] that coined this term in 2007 and since then it has been used and accepted by the literature.

Several anatomical studies failed to clarify if the ALL is present in all knees: Helito et al.[10] reported as the ALL was clearly observed during the dissections of all 20 knees studied and similar data are showed by Claes et al.,[11] who conducted a cadaveric evaluation of the knee structures. This study found a distinct ligamentous structure on the anterolateral side of the joint in all but one of the 41 dissected knees (97%); a lower rate of incidence was reported by Dodds et al.,[12] who identified a consistent structure identifiable as the ALL only in 33 on 40 knees (83% of cases) dissected.

When it was identified, the ALL has been described originating proximally from the prominence of the external femoral epicondyle, slightly anterior to the origin of the external collateral ligament, and proximal and posterior to the insertion of the popliteal tendon.[13] This structure has an oblique course, with a solid anchor to the external meniscus, getting in contact with the inferior-lateral geniculate artery and vein; it takes insertion on the tibia, between the Gerdy's tubercle and the apex of the fibular head, completely separated from the ileum-tibial band.

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Quantitative analysis[11] reported a mean length of the ALL as 41.5 ± 6.7 mm in neutral knee axial rotation and at 90° of flexion and 38.5 ± 6.1 mm in full extension. The mean width was 8.3 ± 2.1 mm on its femoral origin and 11.2 ± 2.5 mm on the proximal tibial insertion: between these, the ALL narrowed near the joint line with an average width of 6.7 ± 3.0 mm.

An important biomechanical role of the ALL as stabilizer of the knee has been hypothesized by Woods et al.[14] in 1979. This study described for the first time a correlation between the Segond fracture and the presence of a significant rotational instability of the knee after anterior cruciate ligament (ACL) injuries. Nowadays is well known that patients with complete ACL tears may have different degrees of rotational instability and an isolated ACL reconstruction may not entirely restore a normal rotatory control leading to a residual laxity.[10] A residual pivot-shift may be observed approximately in 7% of patients after ACL reconstruction even in a scenario of correct positioning of the femoral and tibial tunnels.[15]

Parsons et al.,[16] in a recent anatomic investigation on eleven cadaveric knees, confirmed an important contribution of the ALL as stabilizer of the knee during the internal rotation at flexion knee angles greater than 35°. For this reason, in the recent literature as well as in clinical practice, ALL has begun to acquire relevance in the ligament reconstruction, and it seems that his traumatic rupture could lead to a major instability, which could affect the outcome of the ACL reconstruction itself. Theoretically, the reconstruction of ALL with autograft in addition to the reconstruction of the ACL might help to limit this residual rotatory instability.[17]

Finally, concerns have been raised about the histological composition of the ALL: some authors have identified the ligament as an independent anatomical structure, while others have described it as part of the ileo-tibial band[18] or as a simple capsular thickening.[19]

The goal of this study was to conduce a histological analysis of the ALL on a small portion of this ligament harvested, when identified, performing a total knee arthroplasty as described by previous authors.[4] The novelty of this study was to compare the ALL histological quality with those of a portion of ACL and a portion of capsule, collected by the same patient in order to evaluate and eventually detect specific differences.

The primary endpoint of this study was to understand if the histological structure of ALL is more similar with the ACL or with the anterolateral capsule assessing its nuclear structure, the type of collagen, the presence of adipose tissue, the orientation of the fibers and the cellularity in the samples. The secondary objective was the evaluation of the quality of the ALL elastic fibers and the presence of nerve fibers in its mid-substance and to recognize specific proprioceptive structures in the samples.

2. Materials and methods

Twenty-five consecutive patients (18 Females and 7 Males) scheduled for a total Knee Replacement (TKA) have been enrolled in this study; the average age of the patients was 72,5 years (from 64 to 81) with an average BMI of 28.7 (from a min of 23.5 to a max of 37.3). A single surgeon performed all interventions from February 2016 to June 2016 and the current authors proceeded to the data collection after authorization of local Ethics Committee, and after concession of written consent of the processing of personal data and biological sampling by each patient.

A primary advanced tri-compartmental knee osteoarthritis was the diagnosis in all patients: patients with previous surgical treatment around the knee potentially affecting the anterolateral compartment as a lateral closed wedge osteotomy or surgical treatment for traumatic injuries were all excluded from the study. Patients with an ACL injury, treated or not surgically, have been also excluded.

The surgical approach was performed thought a standard midline skin incision and a medial peripatellar capsulotomy. With the patella everted, the ALL was observed as a relatively consistent structure in the lateral knee, free from the lateral joint and linking the lateral femoral condyle, the lateral meniscus, and the lateral tibial plateau.[4]

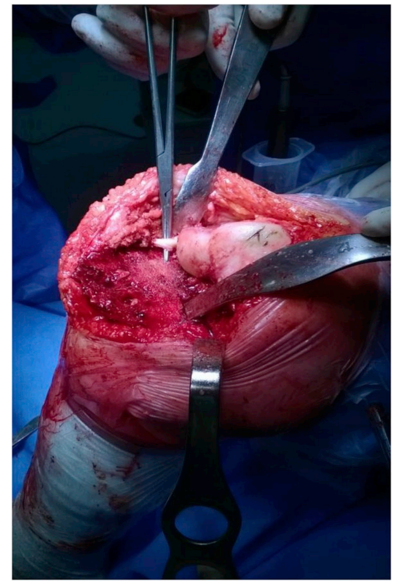

Fig. 1. Anterolateral ligament dissection on the right knee.

During TKA surgery, the authors always tried to identify this anatomical structure and its close relationship with the lateral meniscus in order to be able to take a small specimen from the meniscofemoral portion or from the meniscotibial portion of the ALL itself[11] during the obligatory surgical step of the lateral meniscectomy. A careful dissection was performed to avoid iatrogenic structural damage of the ALL itself (Fig. 1), then a portion of the articular capsule and a portion of the ACL were excised in order to obtain the articular exposure and to proceed with the implant.

All samples have been always harvested following the same surgical protocol: the first sample was collected at the beginning of surgery, during the incision for median parapatellar capsulotomy in medial capsular region (Fig. 2): it appeared as a small fragment of the joint capsule (0.5 cm × 0.3 cm) (Fig. 3). The second sample was the anterior cruciate ligament (Fig. 4), which was then marked with a point of suture at its proximal femoral insertion (Fig. 5). The third sample (0.5 cm × 0.3 cm) (Fig. 7) was taken after the tibial bone resection, in the lateral compartment of the knee, with the articulation at 100° of flexion, internal tibial rotation and patella eversion, collecting a small fragment of ALL as previously described (Fig. 6).

The ALL was identified in all patients (100%) and the structures removed have been sent in separate containers to the pathology service for histological evaluation with the initials of the patient's name, the date of the intervention and indications about the tissue (joint capsule,

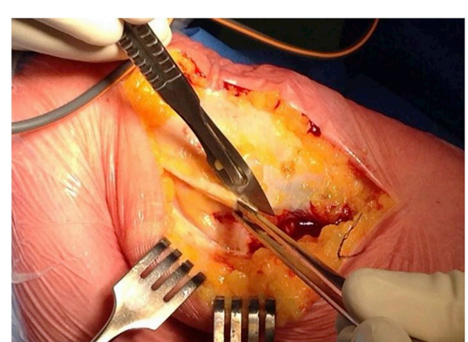

Fig. 2. Donor site of the first sample (joint capsule).

Fig. 3. First sample: fragment of articular capsule.

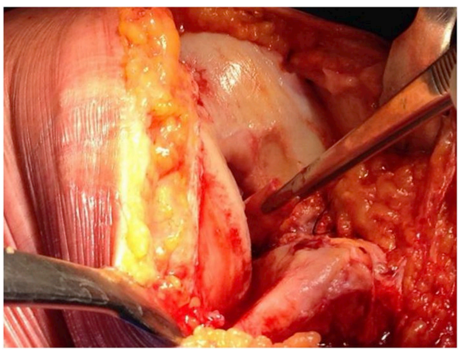

Fig. 4. Donor site of the second sample (anterior cruciate ligament).

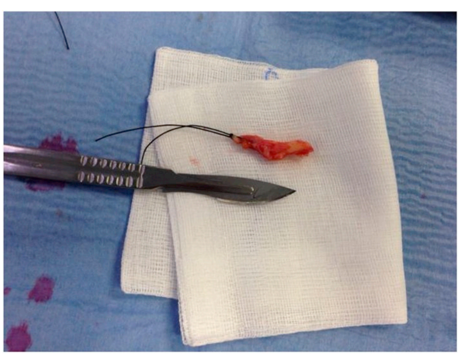

Fig. 5. Second sample: Anterior cruciate ligament fragment.

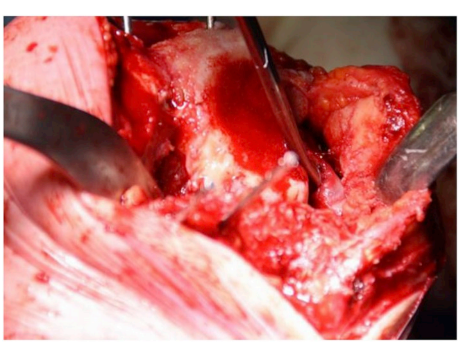

Fig. 6. Donor site of the third sample (Anterolateral Ligament).

anterior cruciate ligament or anterolateral ligament). At this point, each sample was preserved in formalin, sectioned with a microtome and placed on a glass slide with the classic eosin-hematoxylin staining for evaluation of tissue morphology under an optical microscope. For each sample 3 glass slides were prepared (for a total of 75), each with a specific anatomical component and specific histological aspects (Fig. 7 a-c).

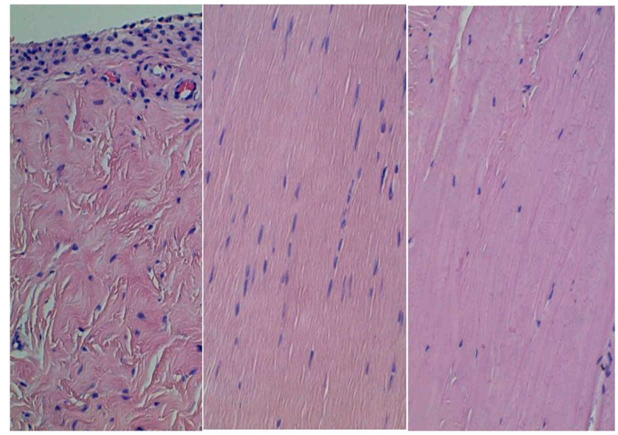

Fig. 7. Hystological samples: from the left side capsule, ACL and AL.

The initial histological analysis allowed to study the morphology for each of the three components taken; in particular we analyzed: the type of collagen present (going to distinguish in lasso or dense), the orientation of the collagenous fibres or their absence, the cellularity of the sample, the presence of elongated or round nucleus, the presence of fat tissue, and any additional components found at microscopic analysis, such as, calcifications, myxoid degeneration, inflammatory tissue and synovitis villosa.

A second preparation was then conducted to perform immune-histochemical analysis evaluating the innervation of the ALL and comparing it with that one of the ACL and the capsule. The tissues underwent S 100 staining and we obtained 56 glass slides as follow: 8 specimens from capsule, 24 specimens from ACL and 24 specimens from ALL. The presence of nerve fibers was then quantified by the number of analyzed microscopic fields looking for proprioceptive structures in the ALL sample and their incidence when compared to the capsule and ACL-ones.

Finally, a third preparation of 56 glass slides, obtained from the same previous 13 samples, was performed to accurately study the elastic fibers using the Weigert staining, which should color the elastic fibers in dark blue, almost black, all surrounded by the remaining colorless structures; the same staining gave negative result (QUESTO VA' INCLUSO NEI RISULTATI, NON IN MATERIALE E METODO) for each of the samples analyzed, demonstrating a non-uptake by elastic fibers.

3. Results

Statistical analysis has been performed using the chi-square test for the assessment of the significance of dichotomous variables, while we have chosen the Student's *t*-test evaluating linear variables.

After histological evaluation of the nuclei, the current authors have not obtained a significant result with the chi-square test considering their form: the presence of 18 round and 7 elongated nuclei in the capsule and 16 rounds and 9 elongated nuclei for the ACL were demonstated. No statistical differences have been found comparing the results obtained evaluating the ALL, which showed 18 round and 7 elongated nuclei ($p = 0.77$).

When reviewing the fat tissue analysis, the researchers predisposed a scale from 1 to 4 regardind the absence or abundance of fat tissue in each sample analyzed, in order to obtain quantitative statistical analysis. It was observed a significant difference among the three anatomical components: this difference, however, was higher between the ALL and the capsule than between the ALL and the ACL. The assessment of the collagen type in the various preparations, such as in the previous nuclei analysis, was conducted using the chi-square statistical test, that has not showed a statistically significant difference among the three structures, highlighting the presence of dense tissue in 20 out of 25 capsule samples, in 19 out of 25 ACL samples and in 23 of 25 ALL samples (p = 0.29).

The orientation of the fibers in the various components has been highlighted using the student's *t*-test too and it demonstrated a statistically significant difference between the capsule (in which there was an orientation in 4 out of 25 samples) and the ALL (in which there was orientation in 8 out of 25 samples); vice versa the analysis of the ACL showed values (presence of fiber orientation in 9 of 25 samples) very similar to the ALL (p = 0.03).

The analysis on the cellularity was performed setting values from 0 (poor) to 3 (abundant), and then statistically evaluated according to the student's *t*-test, which showed no statistical significance between the samples. At the end, using the S 100 staining, we assessed the presence of nerve fibers within the various components and made a statistical evaluation with the student *t*-test, after standardizing the values for the analyzed slide fields, in order to obtain a scale from one to ten. The analysis showed again a significant difference between the ALL and the capsule (p = 0,043), difference not found in the comparison with the ACL (p = 0.38).

A gender difference was examined for each analyzed histological components: the results showed no statistically significant difference, regarding the study of nuclei, the orientation of the fibers and the type of collagen. No significant differences were obtained also for the adipose tissue components and cellularity present in the samples.

4. Discussion

In 1967 Lemaire[19] described the fascia lata reconstruction using an extra-articular tenodesis technique as additional procedure to combine with ACL reconstruction treating chronic ACL deficient knee. Over the years, surgeons have been trying to find solutions for an increasingly less invasive and more anatomical reconstruction of the lateral compartment of the knee: a different anterolateral tenodesis, described by MacIntosh in 1979,[20] was found able to abolish a positive anterolateral jerk test in most knees with a residual rotational instability.

With the increasing renewed interest in anterolateral stabilizing structures, different authors recently described combined intra- and extraarticular reconstruction techniques.[21] According to Marcacci et al.[22] technique, the hamstring tendons harvested leaving their distal insertion are passed through a tibial tunnel and in an over-the-top arrangement on the femoral side. The tendons are then fixed with double staples in the groove, and their remaining part is fixed distally to Gerdy's tubercle. More recently, in 2013, Saragaglia et al.[23] published a similar technique, where the hamstrings are harvested and left attached distally. At the level of the intraarticular portion, the semitendinosus is doubled while the graft exits via a lateral out-in transcondylar tunnel, inferior to the proximal insertion of the lateral collateral ligament (LCL) and tensioned towards Gerdy's tubercle where it can be attached to the fascia lata.

Over the years, the pursuit for a suitable technique able to reconstruct the ALL, restoring the stability of the knee after an ACL lesion, demonstrates the central role of this structure on lateral side of the knee on the rotational instability.

The current authors tried to clarify in this study an unclear aspect of this structure as the histological properties: a key feedback received from our in vivo study is that the anterolateral ligament was found in all 25 cases analyzed, and that is clearly defined by a bundle of fibrous tissue located in the lateral compartment of the knee joint, whose proximal origin is in the lateral femoral condyle and the distal insertion is slightly below the posterior tibial articular surface at the level of Gerdy's tubercle.

We tried to understand if the ligament was differentiated and independent from the anterolateral capsular structure or not: the histological analysis of the three components sampled (capsule, ACL and ALL) proved that, in all patients, the ALL is a distinct structure of dense collagen tissue, constantly present. Previous studies showed a wide variability concerning the presence of this component, its location, and its constitution.[24]

Our study aims to be an “in-vivo” study, which is crucial for some critical detectable points. First, the sample size is fairly limited: we chose a study period of four months, but it is a surmountable element because of the statistical analysis that allowed us to prove the reliability of the results obtained. Secondly, the ethical aspect: being an in-vivo study, it has requested an accurate approach on patients undergoing TKA for not causing any damage or instability correlated to the ongoing study.

The surgical team (always the same in the 25 cases of the study) paid remarkable attention during surgery in the sampling procedure of the various components, to enable a correct histological analysis without affecting the results, avoiding to harvest generous fat component present in the samples area. Similarly, during the histological analysis, we tried to focus on the main fields without considering the outermost portions of the glass slide, often more “contaminated”.

The last factor to be taken into consideration has been the wide confirmation to each histological analysis: being the in-vivo study based on patients enrolled for TKA, the presence of additional elements (calcification, inflammatory cells and myxoid degeneration) determined by the underlying disease might have affected the study results. In particular, we noticed that, where the original joint disease was a severe knee OA, the histological structures showed more additional components during glass slide.

Based on the results we have obtained, we can suggest that the anterolateral ligament shows anatomical and histological characteristics different from the joint capsule and more similar to the native ACL.

5. Conclusion

Our study in vivo demonstrates the constant presence in the knee of the anterolateral ligament. The histological analysis showed that, compared to the capsule, the ALL presents a more architectural orientation of the collagen fibers, an inferior amount of adipose tissue and a inferior amount of proprioceptive type structures. Instead, when compared to the anterior cruciate ligament, it showed a more similar orientation of the collagen fibers, of the nuclei's form, presence of proprioceptive structures and similar cellularity in the samples.

Conflicts of interest

Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jor.2019.03.019.

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