



Influence of anterolateral ligament injuries on stability and second-look arthroscopic findings after allograft transtibial anterior cruciate ligament reconstruction

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ABSTRACT

Background: The purpose of this study was to evaluate the influence of anterolateral ligament (ALL) injuries on stability and second-look arthroscopic findings after anterior cruciate ligament (ACL) reconstruction.

Methods: One-hundred and nineteen consecutive patients underwent a second-look arthroscopic surgery after ACL reconstruction and magnetic resonance imaging (MRI) examination of the ALL. The patients were divided into an ALL intact group ($n = 39$) and ALL injured group ($n = 80$). The ALL injuries were divided according to the three anatomical parts of the ALL (femoral, meniscal, and tibial) using MRI evaluation. Stability and clinical results were evaluated using the Lachman test, pivot-shift test, KT-2000 arthrometer, and Lysholm score. On second-look arthroscopy, graft tension and synovial coverage were evaluated.

Results: The clinical evaluation revealed no significant differences in ALL injury. Although the synovial coverages showed no significant difference ($P = 0.113$), the second-look arthroscopic findings indicated that tension was statistically significantly dependent on the ALL injury ($P < 0.001$). In addition, according to the location of the ALL injury, femoral, tibial, and combined ALL injuries showed significant differences in graft tension as compared with the ALL intact group; only the meniscal injuries had no effect on graft tension.

Conclusion: Combined ACL and ALL injuries showed poor graft tension in the second-look arthroscopic findings after allograft transtibial ACL reconstruction, even though no significant differences in clinical outcomes and stability were observed.

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1. Introduction

Recently, several research studies have reported on the anterolateral ligament (ALL) of the knee. The femoral origin of the ALL is located on the lateral epicondyle and the insertion is about one centimeter distal to the tibial joint line and proximal to the tibial insertion of the biceps femoris [1]. The ALL provides significant anterolateral rotational control during the simulated pivot-shift test and that reconstruction, when combined with intra-articular anterior cruciate ligament (ACL) reconstruction, may be beneficial [1,2].

ACL reconstruction is generally a successful procedure with long-term outcomes of improved function and reduced meniscal injuries as compared with the unreconstructed knee [3]. However, the desire to control rotational instability led to the development of double-bundle reconstructions and, now, increased interest in extra-articular reconstruction [4,5].

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In the history of ALL reconstruction, the first attempts were to restore stability to a knee with an ACL injury [6]. However, the focus continued to shift to intra-articular ACL reconstruction, and extra-articular ALL reconstruction was forgotten by orthopedic surgeons for several decades. Although there has been an improvement in knowledge and surgical techniques of ACL, failure of ACL reconstruction and rotational instability are still issues [7]. As a result, orthopedic surgeons have recently started to reconsider ALL reconstruction to restore knee stability.

In a study where the stabilizing structures of the knee were sequentially sectioned, the anterolateral structures appeared to have the largest role in controlling rotational stability [2]. Furthermore, Sonnery-Cottet et al. [8] reported that the rate of ACL graft failure with additional ALL reconstruction was less than that with solitary ACL reconstruction. Whereas, Claes et al. [9] characterized abnormalities in the ALL in individuals with ACL injury before they underwent surgical treatment and did not differentiate between acute, subacute, and chronic injuries.

Therefore, the purpose of this study was to evaluate the influence of ALL injuries on stability and second-look arthroscopic findings after ACL reconstruction. The hypothesis of this study was that ALL injuries are associated with graft tension and stability after ACL reconstruction.

2. Materials and methods

2.1. Subjects

This study received approval from our institutional research ethics committee, and informed consent was obtained (DKUH 2017-05-007). From 1 January 2009 to 31 November 2016, a total of 253 consecutive patients underwent arthroscopic ACL reconstruction with a modified transtibial technique using an allograft, by a single surgeon (H.G.P.).

The inclusion criteria were as follows: patients who underwent a second-look arthroscopic surgery after primary single-bundle ACL reconstruction using a modified transtibial technique with an allograft and found to have an ALL on magnetic resonance imaging (MRI) performed at our institution four weeks after the initial trauma event. Patients who had a history of previous surgery on the injured knee, multi-ligament injuries, or arthritic changes were excluded.

Among the 253 patients who underwent ACL reconstruction, 27 (10.7%) were lost to follow-up and 18 (7.1%) underwent an MRI examination at other hospitals and thus were excluded. Of the 253 patients, 208 (82.2%) underwent a second-look arthroscopic operation. Among the 208 patients, 40 (19.2%) whose ALLs were not visible on MRI and 49 (23.6%) with >4 weeks' interval since the trauma event were excluded. Overall, among the 208 patients who underwent ACL reconstruction during the study period, 119 met the inclusion criteria. The flow of the patients is demonstrated in Figure 1. The final study population comprised patients with intact and injured ALLs.

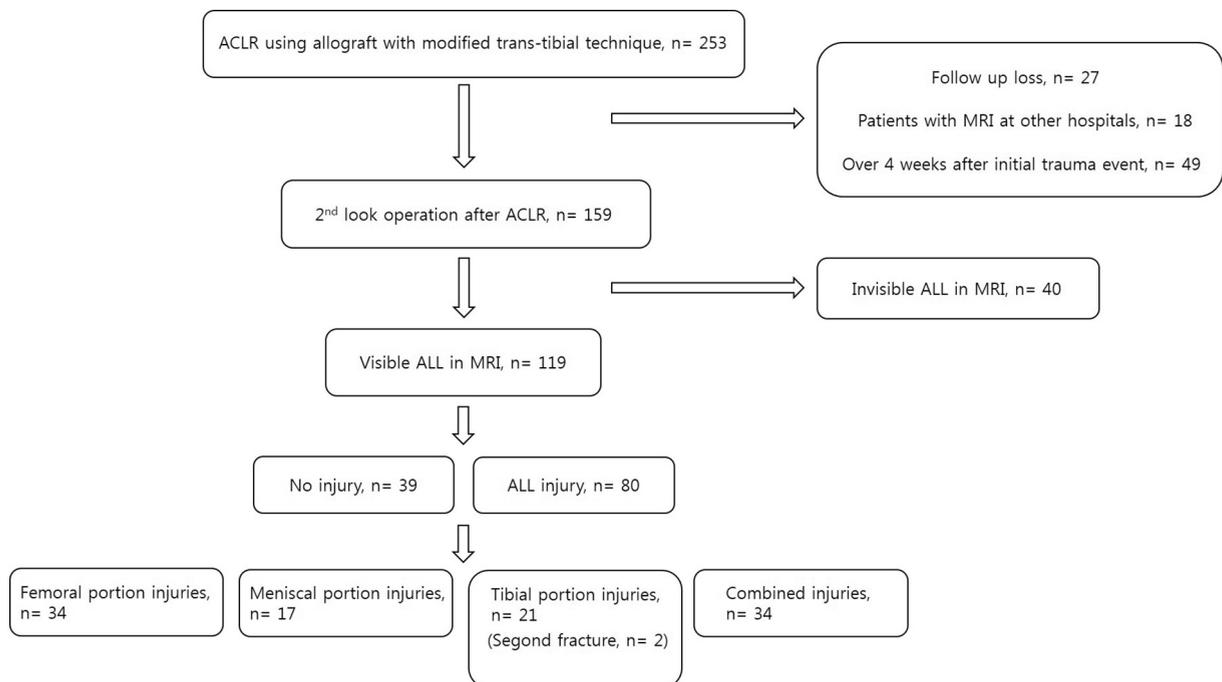


Figure 1. Patient flow throughout the study.

2.2. Surgical techniques (modified trans-tibial technique with allograft)

The tibial tunnel was drilled from the proximal and medial aspects of the tibia to lower the obliquity of the femoral tunnel, using the modified method instead of the conventional transtibial technique. Generally, the starting point of the tibial tunnel is located midway between the posterior cortex of the proximal tibia and the medial margin of the tibial tuberosity. To avoid complete removal of the remnant ACL at the tibial attachment site and prevent impingement of the grafted ACL, minimal notchplasty was performed. Then, an appropriate procedure for the accompanying meniscal or cartilage injury was performed, if necessary. Following the tibial tunnel creation, a transtibial femoral tunnel guide was inserted, and the rear angle of the guide was placed in the 10:30 or 1:30 directions, in the right and left knees, respectively. Then, a tunnel with a depth of 30 mm and a diameter of one millimeter less than that of the graft was drilled to achieve approximately one to two millimeters of the femoral posterior wall. The tunnel with the final diameter was completed using a dilator with an interval of 0.5 mm, and the graft was fixed using the Rigidfix technique (Depuy Mitek, Inc., Raynham, MA, USA). The tibial tunnel was fixed again by using a post-tie after it was fixed with a bioabsorbable interference screw (Arthrex, Naples, FL, USA).

2.3. Postoperative rehabilitation

The postoperative rehabilitation protocol was the same in all the three groups. Joint flexion and extension were allowed starting from the day after surgery. Patients who did not undergo a meniscal repair procedure were allowed to perform partial weight bearing for two weeks while wearing an ACL brace. After two weeks, full weight bearing was allowed. In patients who had a meniscal repair, partial weight bearing was performed for six weeks and the brace was worn for six weeks. Jogging was allowed three months after surgery. Sports activity was allowed six to nine months after surgery depending on the state of recovery.

2.4. Radiological evaluation

MRI evaluations were performed using 3.0-T devices (Philips Achieva, The Netherlands). Our MRI protocol included a coronal T1-weighted sequence; sagittal, axial, and coronal T2-weighted sequences with fat saturation; and a sagittal proton density-weighted sequence. The position of knee in full extension was recommended; however, if the patient was unable to fully extend the knee due to swelling or pain, a slightly flexed position was allowed.

Examination results were assessed by two orthopedic surgeons (J.S.Y. and S.H.K.) who had experience in musculoskeletal disorders. The orthopedic surgeon was considered the main assessor. The evaluation by the orthopedic fellow was used to assess the interobserver correlation.

The ALL injuries were divided according to three anatomical parts: the femoral, meniscal, and tibial portions (Figure 2). Each portion was marked as not visualized, intact, or injured. ALL injuries were considered when proximal, meniscal, or distal detachment; discontinuity of ALL fibers; or irregular contour associated with periligamentous edema was observed. We classified the ALL as normal or as having a lesion.

2.5. Second-look arthroscopic evaluation

All the patients underwent a second-look arthroscopy and hardware removal at least two years after ACL reconstruction. Initially, the ACL graft status was evaluated by a single surgeon (H.G.P.). We used a modification of the classification system for ACL grafts described by Chun et al. to evaluate the tear of graft bundles during second-look arthroscopy [10]. In accordance with this system, graft tear status was graded as normal (probing, <2 mm), lax (probing, >2 but <5 mm), partial tear (probing, >5 mm), and total tear (Figure 3). In addition, synovial coverage of reconstructed grafts was graded as good (nearly entirely covered), half (>50%), and pale (no coverage; <50%; Figure 4). The hardware was removed after the ACL graft evaluation. After the operation, a compression bandage was applied without a splint and weight-bearing was allowed. Running and return to sports were allowed two weeks after the operation.

2.6. Clinical evaluation

Each knee was clinically evaluated before the initial and second-look arthroscopic surgery. A KT-2000 arthrometer test at 30 lb. was performed to measure the side-to-side difference in anterior translation with the knee positioned in 20° of flexion. In the pivot-shift test, the knee was graded as normal, close to normal, and abnormal. The Lachman and pivot-shift tests were performed by a single surgeon (H.G.P.). The Lysholm knee score [11] was used for general evaluation of the knee.

2.7. Statistical analysis

The Kolmogorov–Smirnov test was performed to determine the normal distribution of the continuous data, the continuous variable was analyzed using an independent *t*-test, and the noncontinuous variable was analyzed using Pearson's chi-squared test. Analysis according to the ALL injured portions was performed using the Kruskal–Wallis test, and the post hoc test was performed using the Mann–Whitney test. Interobserver reliability was classified according to the *k*-coefficient as follows: 'slight



Figure 2. Magnetic resonance coronal T2-weighted image with fat saturation depicting an anterolateral ligament. (a) Femoral lesion (arrow); (b) meniscal lesion (arrow); (c) tibial lesion (arrow); (d) combined injury 1: femoral (arrow) and meniscal lesions (arrowhead); (e) combined injury 2: tibial (arrow) and meniscal lesions (arrowhead).

agreement', 0.00–0.20; 'fair agreement', 0.21–0.40; 'moderate agreement', 0.41–0.60; 'substantial agreement', 0.61–0.80; and 'almost perfect agreement', 0.81–1.00. All statistical analyses were performed using the Statistical Package for Social Sciences version 20.0 (SPSS, Inc., an IBM Co., IL, USA). Statistical significance was considered at a *P*-value of <0.05 for all the analyses.

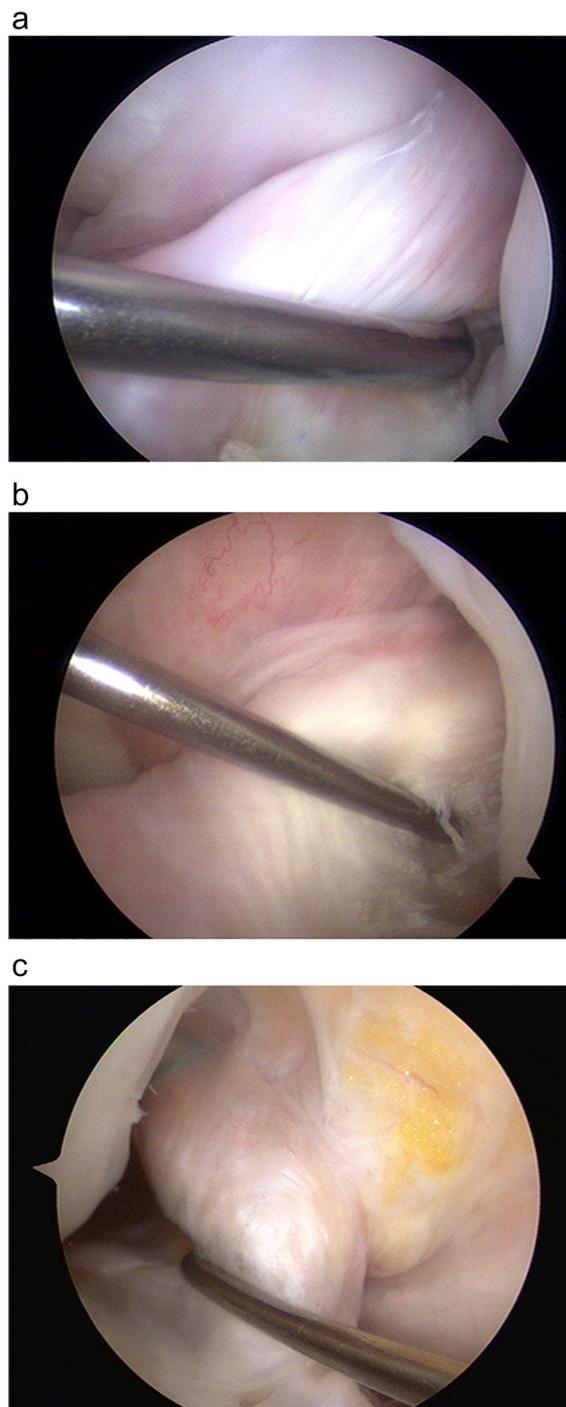


Figure 3. Arthroscopic classification of graft tension. (a) Normal tension of the grafted tendon (probing, <2 mm); (b) lax tension of the grafted tendon (probing, <5 mm); (c) Partial tear of the grafted tendon (probing, >5 mm).

3. Results

With respect to the MRI evaluation of ALL injuries, interobserver reliability (J.S.Y. and S.H.K.) was high, with “almost perfect agreement” ($k = 0.82$). Retrospective power analysis determined that 18 patients were needed in each group to obtain a 10% difference in graft failure between the groups with an α level of 0.01 and a β value of 0.95. In the comparison, the sample sizes of the ALL intact group and ALL injured group were sufficient; however, subgrouping according to the location of ALL injuries did not have a sufficient sample size with an α -level of 0.01 and a β -value of 0.95.

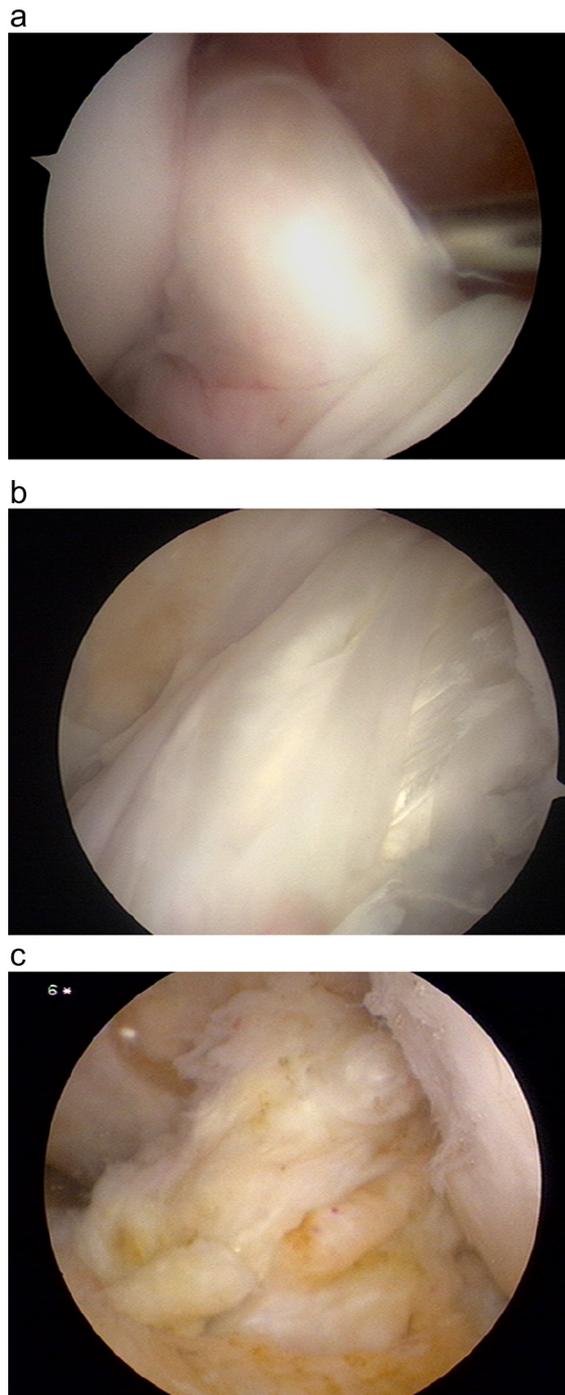


Figure 4. Arthroscopic classification of graft synovial coverage. (a) Good synovialization of the grafted tendon (nearly entirely covered); (b) half synovialization of the grafted tendon (>50%); (c) pale synovialization of the grafted tendon (no coverage; <50%).

3.1. Demographic data

The mean age in the ALL intact group was 30.6 years (range, 20–40 years). Of the patients, 84.6% ($n = 33$) were male and 15.4% ($n = 6$) were female. The mean age in the ALL injured group was 28.9 years (range, 18–40 years). Of the patients, 87.5% ($n = 70$) were male and 12.5% ($n = 10$) were female. Follow-up second-look arthroscopy was performed at a mean of 25.3 months (range, 24–28 months) for the ALL intact group and 25.6 months (range, 24–29 months) for the ALL injured group. No significant differences in age, sex, weight, height, body mass index, mean duration to follow-up second-look arthroscopy, graft

tendon thickness, type of allograft, location of meniscal tears, and surgical treatment for the meniscus were found between the groups (Table 1).

3.2. Comparison of clinical outcomes in association with ALL injury

The mean Lysholm score was 93.3 ± 2.1 (mean \pm standard deviation) in the ALL intact group and 93.2 ± 2.2 in the ALL injured group, which showed no statistically significant difference ($P = 0.778$; Table 2). KT-2000 arthrometer test results at 30 lb, measuring anterior translation at preoperation and last follow-up were, respectively, 4.8 ± 1.4 and 1.4 ± 0.9 mm in the ALL intact group and 4.7 ± 1.7 and 1.4 ± 1.0 mm in the ALL injured group. Both groups showed improved postoperative outcomes as compared with the preoperative status, but no statistical difference was found between the preoperative and last follow-up periods ($P = 0.759$ and 0.833 , respectively; Table 2). The Lachman test revealed negative results in both groups at last follow-up and no statistically significant difference between the groups ($P = 0.180$; Table 2), as in the pivot-shift test ($P = 0.483$; Table 2).

3.3. Comparison of second-look arthroscopic finding in association with ALL injury

Of the 39 patients in the ALL intact group, 31 (79.5%) showed a normal graft, eight (20.5%) showed a lax graft, and none had partial and total tears. Of the 80 patients in the ALL injured group, 10 (12.5%) showed a normal graft, 62 (77.5%) showed a lax graft, eight (10%) showed a partial tear, and none had a total tear. Graft tension in association with ALL injuries showed a statistically significant difference between the groups ($P < 0.001$; Table 3).

In the 39 patients in the ALL intact group, 18 (46.2%) showed good synovial coverage, 21 (53.8%) showed half synovial coverage, and none showed pale synovial coverage. Of the 80 patients in the ALL injured group, 42 (52.5%) showed good synovial coverage, 38 (47.5%) showed half synovial coverage, and none showed pale synovial coverage. Formation of synovial coverage in association with ALL injuries showed no statistically significant difference between the groups ($P = 0.113$; Table 3).

3.4. Clinical outcomes and second-look arthroscopic findings according to the injured portion of ALL

We divided the patients into four groups, namely the femoral, meniscal, tibial, and combined injury groups (injuries in >2 portions), assessed using MRI. Of the 80 patients in the ALL injured group, 34 (42.5%) had a femoral injury, 21 (26.2%) had a tibial injury, 17 (21.3%) had a meniscal injury, and eight (10%) had combined injuries. Interobserver reliability was in almost 'perfect agreement' ($k = 0.82$).

No statistically significant difference was found between the injured portion of the ALL and the clinical outcomes and stability at last follow-up. The second-look arthroscopic findings showed that synovial coverage had no significant difference ($P = 0.385$). Although graft tension showed significant poor laxity in the ALL injured group ($P < 0.001$), only the meniscal portion had no statistically significant difference ($P = 0.065$; Table 4).

4. Discussion

In the present study, the ALL injuries significantly affected the ACL graft tension in spite of the satisfactory clinical outcomes and stability. To the best of our knowledge, this study is the first to evaluate the influence of ALL injuries on arthroscopic findings after ACL reconstruction.

The ALL functions as a secondary stabilizer to the ACL in resisting anterior tibial translation and internal tibial rotation and in preventing the knee pivot-shift phenomenon [8,12–14]. Most biomechanical studies [13–15] have demonstrated a significant effect of the ALL in providing rotational control of the knee during the simulated pivot-shift, although at least one study [12]

Table 1
Demographic data.

	ALL intact group (n = 39)	ALL injured group (n = 80)	P
Age	30.6 ± 10.1	28.9 ± 10.7	0.410
Sex (male:female)	33:6	70:10	0.665
Weight (kg)	74.3 ± 14.9	75.8 ± 16.9	0.388
Height (cm)	173.3 ± 6.9	172.2 ± 6.8	0.628
Body mass index	24.6 ± 4.3	25.3 ± 5.2	0.371
Mean follow-up to second-look arthroscopy (months)	25.3 ± 4.1	$25.6.6 \pm 9.1$	0.286
Graft tendon thickness (mm)	9.9 ± 0.3	9.8 ± 0.6	0.348
Allograft tendon (Achilles:tibialis)	34:5	69:11	0.307
Medial meniscus tear	11	18	0.715
Lateral meniscus tear	9	22	0.463
Medial and lateral meniscus tear	2	6	0.459
Meniscectomy	8	15	0.299
Meniscus repair	6	8	0.472

ALL, anterolateral ligament.

Table 2
Arthroscopic findings comparison of anterolateral ligament injury.

	ALL intact group (n = 39)	ALL injured group (n = 80)	P
Tension			<0.001
Normal	31	10	
Lax	8	62	
Partial tear	0	8	
Complete tear	0	0	
Synovial coverage			0.113
Good	18	42	
Half	21	38	
Pale	0	0	

ALL, anterolateral ligament.

suggested that the ALL makes only small contributions to restraining internal tibial rotation and that the iliotibial tract is the primary restraint during the pivot-shift test. In ACL-deficient states, the function of the ALL is most important, with most biomechanical studies demonstrating that in the presence of ACL insufficiency, detaching or sectioning the ALL in cadaveric knee specimens results in a significant effect on the anteroposterior (AP) stability and a significant increase in internal rotation [13,15–17]. However, Tavlo et al. [14] found that after ACL reconstruction, no significant difference was found between an intact and a detached ALL in terms of AP knee stability. Furthermore, ALL detachment had a significant effect on internal rotational stability in ACL-insufficient knees but a non-significant effect in ACL reconstructed knee. With regard to ALL reconstruction, Spencer et al. [13] found that in an ACL-deficient state, ALL reconstruction did not significantly reduce internal rotation or anterior translation as compared with that in an ALL-deficient state. In the present study, preoperative and last follow-up clinical outcomes and stability after ACL reconstruction did not show significant differences according to ALL injury. The authors also expected that preoperative clinical stability would be affected by ALL injury, however the results showed no difference not only postoperatively in ACL reconstructed knee but also preoperatively in ACL deficient knee. This can be explained by the selection bias: ACL reconstruction was performed in the cases with persistent instability and stable ACL deficient knees without ALL injury were excluded in the present study.

However, concomitant ACL and ALL reconstructions have been shown to significantly reduce internal rotation and axial plane tibial translation as compared with isolated ACL reconstruction in the presence of an ALL deficiency [18]. Furthermore, Sonnery-Cottet et al. [8] reported that in a high-risk population of young patients participating in pivoting sports, the rate of graft failure with ACL and ALL reconstructions was less than that with ACL reconstruction alone. In this study, arthroscopic graft tension at the second-look arthroscopic examination was significantly poor in the ALL injured group, even though clinical stability showed no significant difference. These results showed that clinical stability did not perfectly reflect graft state, because clinical stability is the result of a combination of various conditions, although graft tension is one of the most important factors.

According to previous studies, characterization of the ALL by MRI of the anterolateral region of the knee is not always possible because of the presence of adjacent structures such as the lateral collateral ligament, popliteus tendon, iliotibial band, and

Table 3
Clinical outcomes comparison of anterolateral ligament injury.

	ALL intact group (n = 39)	ALL injured group (n = 80)	P
Mean last Lysholm score	93.3 ± 2.1	93.2 ± 2.2	0.778
Mean KT-2000			
Pre-operation	4.8 ± 1.4	4.7 ± 1.7	0.759
Last follow-up	1.4 ± 0.9	1.4 ± 1.0	0.833
Lachman test			
Pre-operation			0.391
Normal	0	0	
Close to normal	7	17	
Abnormal	32	63	
Last follow-up			0.180
Normal	33	74	
Close to normal	6	6	
Abnormal	0	0	
Pivot-shift test			
Pre-operation			0.683
Normal	5	8	
Close to normal	7	20	
Abnormal	27	52	
Last follow-up			0.483
Normal	39	79	
Close to normal	0	1	
Abnormal	0	0	

ALL, anterolateral ligament.

Table 4

Clinical outcomes and arthroscopic findings and according to the location of anterolateral ligament injury.

	ALL injured group (n = 80)								P
	Femur (n = 34)	P (comparison with ALL intact group)	Meniscus (n = 17)	P (comparison with ALL intact group)	Tibia (n = 21)	P (comparison with ALL intact group)	Combined injuries (n = 8)	P (comparison with ALL intact group)	
Last follow-up									
Lysholm score	93.0 ± 1.9	0.994	93.6 ± 2.1	0.998	92.8 ± 2.9	0.940	94.2 ± 7.5	0.807	0.587
KT 2000	1.4 ± 0.9	1.000	1.2 ± 0.9	0.998	1.4 ± 1.3	1.000	1.6 ± 0.5	0.997	0.959
Lachman test		0.996		0.891		0.791		0.929	0.682
Normal	30		16		20		8		
Close to normal	4		1		1		0		
Abnormal	0		0		0		0		
Pivot-shift test		1.000		1.000		0.398		1.000	0.464
Normal	34		17		20		8		
Close to normal	0		0		1		0		
Abnormal	0		0		0		0		
Tension		<0.001		0.065		<0.001		<0.001	<0.001
Normal	3		6		1		0		
Lax	28		11		16		7		
Partial tear	3		0		4		1		
Complete tear	0		0		0		0		
Synovial coverage		0.549		0.860		0.351		0.519	0.385
Good	17		8		12		5		
Half	17		9		9		3		
Pale	0		0		0		0		

ALL, anterolateral ligament.

anterolateral capsule, which cause a partial volume effect in the region, hampering the identification of that structure [19]. Recently, ultrasound has also been attempted for ALL evaluation. Cavaignac et al. [20] reported that the ALL was identified in all patients using ultrasound, whereas MRI identified the ALL in 96% of patients.

However, the small ALL thickness and its anatomical variability also make complete visualization difficult in MRI [21–23]. Helito et al. [21] performed a study in 39 patients and identified the ALL in its entirety in approximately two-thirds of the MRI scans, but additional coronal proton density-weighted sequences without fat saturation were also used. Taneja et al. [24] reported a study of 70 patients in which only a low percentage of MRI scans showed the ALL in its entirety and 50% of the scans showed some portions of the ligament. Porrino et al. [25] examined MRI scans of 53 patients and, in contrast to other studies, were able to characterize the ALL in its entirety in all of the MRI scans. Notably, Porrino et al. [25] performed their tests on a 3.0-T device, whereas Helito et al. [21] used a 1.5-T device. Even when using 3.0-T devices, the ALL could not be characterized in all examinations. Future studies using less spacing between images, smaller slice thickness, volumetric sequences, and special sequences focused on the ALL path should be conducted to increase the accuracy of the visualization of the ALL. In the study of Claes et al. [9] the ALL was not characterized in 24% of the evaluated knees, a percentage similar to that found by Helito et al. [21] in knees of patients without injury and slightly higher than that in the present study. In our study, 119 (74.8%) of 159 cases could be characterized, although a 3.0-T MRI device was used. With regard to the location of the ligament injuries and the number of observed abnormalities, Claes et al. [9] found that of all the ACL injuries, 77.8% (162/208) were combined ALL injuries. De Maeseneer et al. [22] reported that ALL injuries accounted for 76.9% (10/13) of all ACL injuries. The present study showed a similar incidence of ALL injury in patients with ACL injuries (67.2%, 80/119).

Helito et al. [19] reported 33 cases of ALL injury, of which 72% were proximal injuries, 21% were tibial injuries, six percent were combined proximal and distal injuries, and 48% (16 cases) were meniscal injuries. Our study showed 34 cases (42.5%) of proximal injury, 17 cases (21.3%) of meniscal injury, and 21 cases (26.3%) of tibial injury among the 80 cases of ALL injury; 34 cases (42.5%) were combined proximal and meniscal or distal and meniscal injuries. Segond fractures were reported in 65.6%, 42.8%, and 9.5% of the tibial injuries in the study of Claes et al. [26] and Helito et al. [19], and in our study, respectively. Fernandes et al. [27] reported that patients with Segond fractures had increased persistent instability after ACL reconstruction because of Segond fracture caused by avulsion of the tibial attachment of the ALL. In our study, only meniscal injury did not show a statistically significant effect on graft tension. Graft tension is less affected by meniscal injury because bone-to-bone contact is maintained when the proximal and distal portions are intact. However, the *P*-value of the relation between meniscal injury and graft tension was 0.065. Therefore, a type 2 error due to a small sample size is possible due to the distribution of ALL injuries according to the location of injury.

This study has several limitations. First, no gold standard modality was used for the characterization and comparison of ALL injuries suggested by MRI findings. Although MRI is a method with great potential, the injury criteria were established arbitrarily on the basis of the experience of the authors and previous studies that evaluated this structure [3–5,7–10,17,28]. Second, the lack of association between the location of the ALL and the instability might have been a source of a type 2 error due to the sample size used, and this should also be considered as a limitation of this study. Third, the effects of ALL injuries on ACL reconstruction

failure could not be analyzed. In this study, none of the cases showed total rupture of the ACL graft because all failure cases were excluded in accordance with the exclusion criteria.

5. Conclusion

Combined ACL and ALL injuries showed poor graft tension in the second-look arthroscopic findings after allograft transtibial ACL reconstruction, even though no significant differences in clinical outcomes and stability were observed.

Conflict of interest

The authors have no conflicts of interest to declare.

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