Clinical and experimental evaluation of several grafts utilized for ACL reconstruction

PhD Thesis

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Clinical Medical Sciences D94 B-1/2010

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Pécs 2016

Abbreviations

ACL	anterior cruciate ligament
AM	anteromedial
AP	antero-posterior
BTB	bone - tendon – bone
DSC	differential scanning calorimetry
I	intermedier
IKDC	International Knee Documentation Committee
KOOS	Knee injury and Osteoarthritis Outcome Score
LFC	lateral femur condyle
MFC	medial femur condyle
MRI	Magnetic Resonance Imaging
PCL	posterior cruciate ligament
PL	posterolateral

Aims

The aim of the present dissertation is to examine the results of anterior cruciate ligament (ACL) reconstruction procedures carried out in everyday practice in terms of the tendon grafts used. With the rapid development of surgical technology, both the surgical technique both the instruments used during medical care is in a constant change and is becoming more and more sophisticated. The grafts used during the operation, on the other hand, are constant with a small variability, even though there is freedom of choice. We aimed to explore with our clinical and academic research (histology, differential scanning calorimetry (DSC)), what effect graft choice has on our results when the surgical technique, instruments and post-surgical care are standardized. In other words, we expected from the study that upon evaluating the results, the method and time of care could be optimized.

In our study, our goal was to assess the efficiency of treatment in our institute based on a retrospective study on injured who were treated with the same surgical technique, and had post-surgical care based on the same protocol, but received different grafts for the replacement of ACL.

During the survey, we compared the functional stability and muscle strength of the lower limb of patients who received different grafts with ones who refused the operation. In view of the results of the objective investigation, we were interested to know, how the different patient groups rate their own condition subjectively. We used two questionnaires to do that, KOOS and IKDC.

Since there is a tissue bank at our university, we have the opportunity to use allografts during the ligament replacement surgery that were deep freezed and stored at -80 °C. With carried out DSC examinations, to answer the question, how deep freezing influences the histological structure of tendons and this is related to the time elapsed between donation and utilization. At the same time, we also examined the samples with conventional histological methods, based on which we aimed to find a correlation between microscopic tissue and molecular structure changes.

The questions in summary:

Is there a graft used in clinical practice for the replacement of ACL, which is more advantageous in terms of the objective measures of functional results?

Do the objective functional results correlate with the subjective opinion of the patients?

Is it possible to demonstrate time dependent tissue damage in case of the allografts used for the replacement of ACL?

Can an optimal time be defined, until which the allografts can be used safely and with good results?

Introduction

Anatomy of the knee

The knee joint is the largest and most complicated joint of the human body, based on its movements it is trochoginglymus. The stability in every position is provided by the strong ligament system of the joint as a passive, and the muscles as active stabilizators.

The medial stabilizators that prohibit the opening of the knee in the valgus direction are the dorsomedial joint capsule, medial collateral ligament, capsule, posterior oblique ligament, the muscles that belong to the pes anserinus and the semimembranous muscle.

The lateral stabilizators are the dorsolateral capsule, lateral collateral ligament, popliteal muscle, iliotibial tract, and the biceps femoris muscle.

The menisci and the cruciate ligaments provide the stabilizing system of the knee. The posterior cruciate ligament runs from the lateral side of medial condyle of the femur, from the ventral direction posteriorly and downward to the fossa intercondyloidea posterior tibiae and consists of two bundles.

The anterior cruciate ligament originates from the medial dorsal side of the lateral condyle of the femur, and inserts widely in front of the eminence between the horns of the two menisci (Figure 1). According to the general view, the anterior cruciate ligament

consists of three fiber bundles, which, along their course partly rotate along their own axis as well. In every stage of flexion, a different fiber bundle stretches.

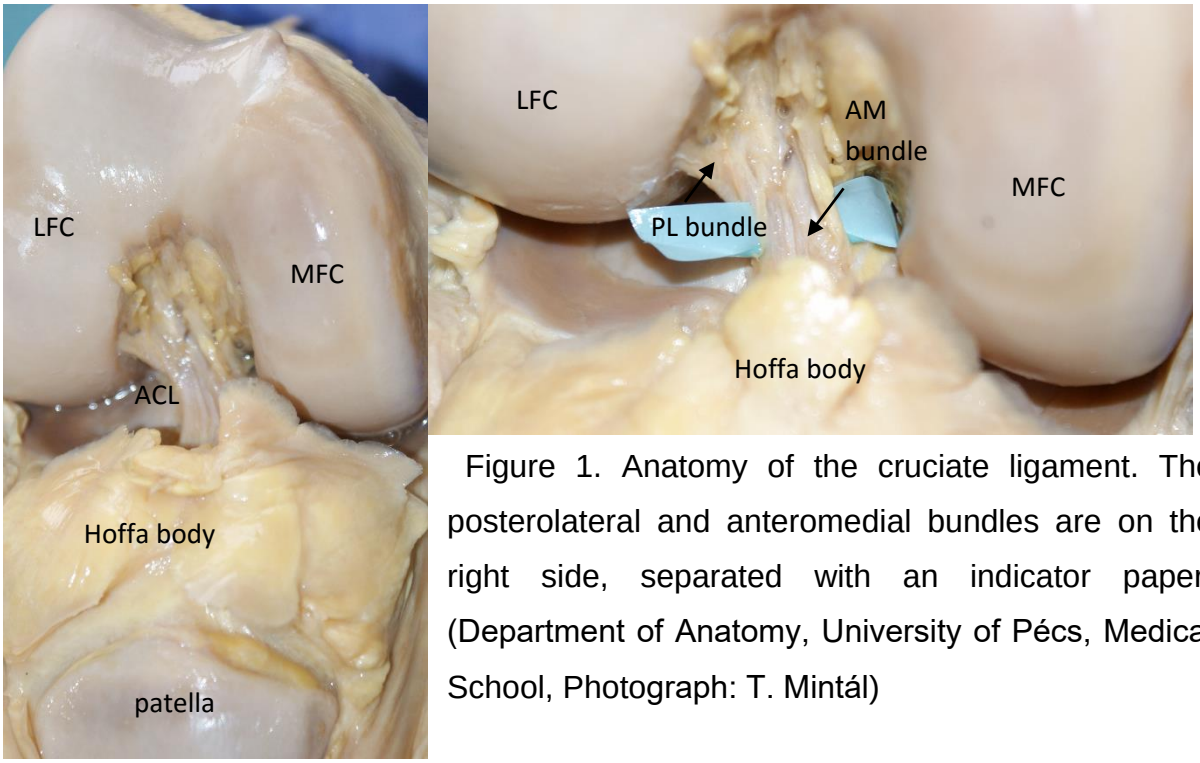

Figure 1. Anatomy of the cruciate ligament. The posterolateral and anteromedial bundles are on the right side, separated with an indicator paper. (Department of Anatomy, University of Pécs, Medical School, Photograph: T. Mintál)

According to studies, the anterior cruciate ligament has 2 or 3 separable bundles that are named based on their insertion on the tibia. Thus, we distinguish a larger anteromedial bundle, which originates more proximally on the femur, and inserts anteromedially on the tibia. On the contrary, the posterolateral bundle as its name states, inserts laterally and more posterior to the AM ligament. There are authors who name a third, intermedier bundle, which they managed to dissect in older patients, while there was only one macroscopic bundle in case of younger patients (Figure 2).

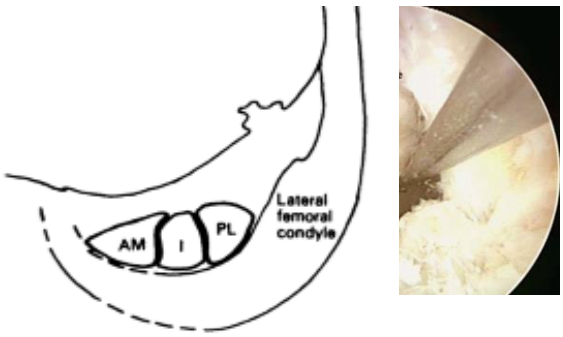

Figure 2. The origin of the anterior ligament in case of two and three bundles.

Christopher Kweon , Evan S. Lederman, Anikar Chhabra Anatomy and Biomechanics of the Cruciate Ligaments and Their Surgical Implications

The functional stability of the knee

The complex relationship of the anterior and posterior cruciate ligaments determines the dynamic stability of the knee. The length and tension of the anterior and posterior cruciate ligaments change constantly during knee movement.

The anteromedial bundle stretches during flexion, while the posterolateral bundle is relaxed. In extension they behave exactly the opposite, the anteromedial bundle relaxes, and the posterolateral bundle becomes stretched.

The dynamic stability depends on the joint geometry, the ligament system and the active stabilization system, or in other words, the muscles. In a joint, where the anterior cruciate ligament is injured, the muscles have a greater role. This serves as the basis of the rehabilitation of injuries that can be treated conservatively. Thus, the movement and the dynamic stability of the joint is directed by the neuromuscular system.

The proprioceptive or in other words sensorimotor system is a complex neurosensory and neuromuscular system. This system defines the basis of all movements, which consists of the system that stabilizes the knee as well, changing constantly and dynamically during normal sports activity. During certain sports activity, however, the latency of this reflex is enough for the cruciate ligament to be torn.

In an injured, torn cruciate ligament, this proprioceptive function is missing, this is the reason for functional knee instability leading to further injuries.
Regaining normal muscle action (function and strength) has to be the major aim of rehabilitation after cruciate ligament replacement surgery. In many cases the weakness of the quadriceps muscle is responsible for the weak knee function, despite rehabilitation.

The anterior cruciate ligament injury

The mechanism of the anterior cruciate ligament tear

The tear of the anterior cruciate ligament can happen due to direct or indirect trauma. The injury caused by direct impact is more rare. Its typical case is when the stretched

leaning knee is kicked from the outside, putting the knee under strong valgus stress, for example during football. In this case, the medial ligament almost always gets torn, as an accompanying injury.
The tear due to indirect impact is more common, when the flexion, inward rotation and valgisation together result in an injury on the standing leg. This is a common ski injury, but not rare in basketball or football either. In this case, besides medial meniscus tear, lateral ligament injury can also accompany the anterior cruciate ligament tear.

The incidence of anterior cruciate ligament tear

The incidence of anterior cruciate ligament tear shows an increasing tendency worldwide. In the United States this means that it increased from 86,687 in 1994 (32.9/100,000 people/year) to 129,836 by 2006 (43.5/100,000 people/year). Interestingly, the increase was the most prominent in the age groups under 20 and above 40. Another notable observation was that the increase was most prominent among women, it increased from 10.36 to 18.06/100000 people/year (P=0.0003). This, among men was only 22.58/100,000 people/year and 25.42/100,000 people/year. Since there is no national register in Hungary, we do not have exact local data. With the development of welfare society, the change of health perception and the transformation of leisure activities, similar case increase can be observed in Hungary as well.

Sex differences

The risk of anterior cruciate ligament injury starts to increase at the age of 13 for girls, and 15 for boys. In case of girls, the occurrence peaks between 15-20 years of age. In case of boys, the peak is sifted to 1-2 year later, reaches its highest in the adolescent age and then starts to decrease. It is an interesting ascertainment, that in the secondary school age, the number of sports related (football, basketball, baseball, volleyball, athletics) anterior cruciate ligament injuries is considerably higher for girls than for boys. This rate levels out in professional sports. Comparing the sexes, they ascertained that women are more accepting regarding surgery, and a lot smaller proportion of them carries on with the previous sports activity after the surgery.

The diagnostics of anterior cruciate ligament tear

The anterior cruciate ligament replacement is routinely performed in orthopedic surgery, and its goal is to restore the functional stability of the knee. Consequently, further injuries, damages can be prevented and the function can improve.

The diagnosis of the anterior crucial ligament rupture follows a well defined scheme. The basis of it is the careful physical evaluation after thorough anamnesis. In case of anterior cruciate ligament rupture, the Lachman-test, the Anterior Drawer test and the Pivot shift test can be informative.

During Lachman test, the patient lies on his back and the knee is bent in 20-30 degrees, we hold the tibia and the distal femur and attempt to move the tibia anteriorly. If the endpoint is soft, the anterior cruciate ligament is probably torn. If we can feel a firm endpoint, that implies that the ligament is intact even when the joints are relaxed. Of course, in every case, the examination result has to be compared with the healthy side.

During the Anterior Drawer test, the knee has to be bent 60-70 degrees, and we provoke the shift of the tibia with regards to the femoral condyles. This test, however is not as accurate, since the stretch of the femoral flexor muscles could hinder the anterior movement of the tibia. It only gives reliable results in case of well cooperating, relaxed patients.

The pivot shift test is more difficult, it requires an experienced examiner. This is also carried out on a patient lying on his back. In the starting position, the examiner fixes the leg in inward rotation, and bends the knee applying valgus stress at the same time. If the tibia subluxes during flexion, the test is positive.

The Appley and McMurray tests serve to detect accompanying meniscus injury.

We examine lateral ligament stability with the varus and valgus stress tests.

Imaging tests

Taking two way axial patella and tunnel records can be justifiable in case LCA tear is suspected. However, direct signs can rarely be seen on them. Such direct sign can be the bony tear from the origin or insertion of the ligament, or the so-called Segond sign, which is the shell like fracture of the edge of the lateral tibia condyle on the AP view. Signs implying chronic tear: pointedness, hypertrophy of the eminentia intercondyloidea, narrowing of the joint gap and fossa intercondylaris.

The MRI is the most sensitive diagnostic method of the anterior cruciate ligament tear. In case of LCA tear, we can see the discontinuation of the ligament and an irregular wavy anterior edge (Figure 3). The anteriorly subluxed tibia, which is the consequence of LCA injury, is indicated by the sharply broken posterior cruciate ligament. There is increased signal intensity in the ligaments on weighed T2 images. There is an accompanying injury with the anterior cruciate ligament tear in 80% of cases, which could justify an MRI scan. It is an expensive and complicated test, and is not necessarily needed alongside a thorough physical examination.

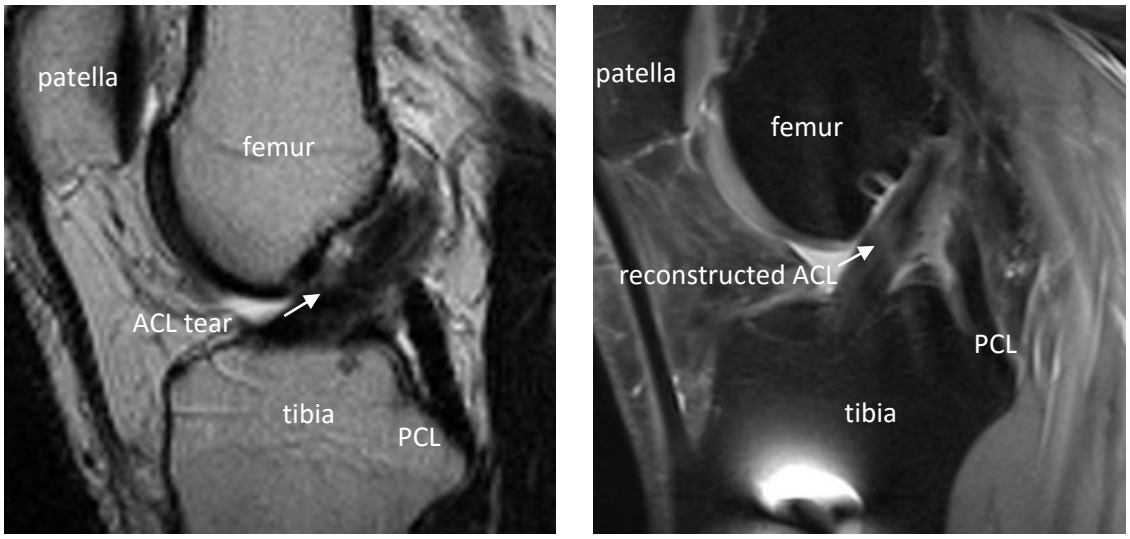

Figure 3. MRI image of anterior cruciate ligament tear and replaced ligament (Source: Pécs Diagnostic Center, Own material).

Treatment of anterior cruciate ligament tear

After a firm diagnosis of anterior cruciate ligament tear has been made, we have to decide regarding the method of treatment. We can treat anterior cruciate ligament tear conservatively or we can perform a surgery following an injury.

Conservative treatment can be effective in part of the patients. This is suggested when, during arthroscopy, the tear is smaller than 50%, and the difference in the degree of instability between the two knees is smaller than 50%, the rate of instability difference between the two knees is smaller than 5mm, and the pivot shift is negative. The most important alongside these is that the patient does not feel that the knee is instable.

Conservative treatment is suggested when the injured is inactive, the compliance is insufficient, there is a knee joint inflammation, or other he has a rheumatological knee disease. Pronounced knee arthrosis is also a reason against surgery, we cannot expect good surgical results in this case. Older age is also a contraindication, but

literature shows that good results can be achieved in a sufficiently active population even above the age of 50. Our experience support this view as well.

If restoring the functional stability of the knee cannot be expected from conservative treatment, or the injured leads an active lifestyle and would like to return to the previous activity level, we recommend surgery.
Choosing the optimal time of operation does not follow a coherent scheme. Our greatest enemy is arthrofibrosis, the risk of which can be minimized by carefully choosing the time of operation. In case of early operation (i.e. within 1 week of injury) we have to expect this complication to take place.
Almekinders et al. have found arthrofibrosis to be three times as common (18%) when the surgery was performed during the first week after injury, compared to when they operated after the 4th week (6%).
Naturally, the time of intervention also depends on the local status of the knee. Hemorrhages, narrowing of movements and weak muscles all indicate delayed operation.

In our practice we perform surgery after the 6th week of injury. In case there is no accompanying injury that requires acute intervention, this period is sufficient for the mental preparation of the patient as well. Since recovery and rehabilitation take a long time, the time before the surgery is suitable for arranging rehabilitation and altering lifestyle as well. Physiotherapy before the operation improves movement range, conditions muscles, and not the least enables the patient to carry on with the postoperative rehabilitation with a well practiced exercise routine not having to learn them with a recently operated painful knee.

Anterior cruciate ligament replacement nowadays is considered a common procedure in institutes performing sports surgeries

With the advancement of surgical techniques, there are many known surgical techniques and instruments. There is an agreement, that nowadays we do not perform no open joint surgery anymore. In view of the well examined biomechanical parameters, the general view nowadays is that the graft should be inserted to the anatomical location. The earlier well analyzed question, how many bundles should we use for the replacement following the fine structure of the cruciate ligament, also seems to become more clear. It seems, the reconstruction with multiple bundles does not have

a notable advantage compared to the previous single ligament replacement, moreover, the possible revision of the former has a potential for much more complications. I am convinced that is the case as well. The other fundamental question in cruciate ligament surgery is the quality of the graft. There are many options for that as well, mostly the opportunities of the surgeon and the needs of the patient settle the choice.

The grafts used for the anterior cruciate ligament replacement

In case of surgery, we have to replace the torn ACL with a suitable graft choice. When choosing the graft, we have to take into consideration the anatomy of the patient, previous surgeries, injuries, sport he aims to do, therefore we can say that it is a multifactorial consideration. The most often used grafts are the autologue hamstring, the patellar tendon (BTB), and the allografts. The former can be posterior tibial tendon, hamstring, BTB and Achilles tendons. Using synthetic grafts is very problematic, therefore, except for a few special indication, their use is not recommended. The advantages of using allografts is that we do not have to expect later complaints at the donor site and rehabilitation can be faster, since we did not disrupt either the extensor or the flexor apparatus. Besides, integration can be slower and more uncertain, infection transfer can occur despite every precaution (0,014-0,00015%), moreover, it is not always and anywhere accessible due to complicated storage. On the contrary, when using an autograft, there is no infection transfer, it is always at hand, but the pathology of the donor area, the decrease in muscle strength and the possible saphenous nerve injury are all counterarguments.

Semitendinous autograft

We search for the semitendinous autograft 2cm medial from the tuberositas tibiae and from an incision 4cm from the line of the joint. The pes anserinus is located here, one member of which is semitendinous, the other is the gracilis tendon, the third is the sartorius. The advantage of this exploration is that we can start the drilling of the tibial tunnel form the same site as well. We look for the tendon of the semitendinous, in the group, and stitch its distal end. We remove it from the periosteum with a tendon stripper. The tendon we have thus retrieved is 20-28 cm long. Folding it 4-fold we get the length and the thickness of our graft. The graft cannot be thinner than 7mm and thicker than 10mm, because that would cause a disproportion in space. If neither the

length nor the thickness of semitendinous tendon is enough on its own, we can also remove the tendon of the gracilis. This is the classic hamstring graft. The tendon after the insertion will grow into the tunnel we have made. This happens slower than the healing to the bone (in case of BTB).

Patellar tendon autograft

When using own patellar tendon, we make a 6-8cm long incision. We usually cut a 10mm piece of appropriate thickness from the middle section of patellar ligament. We remove bony ends from the tuberositas tibiae and patella with an oscillation saw or a special frazer. We can tailor the bony ends to suitable size and shape. From the distal part of the wound, with a bit of difficulty, the planned starting point of the tibial tunnel can be reached. In case of the patella, we have to be careful not to weaken the bony tissue too much because that can lead to fracture.

Allografts

In case of allografts we can use as described before, semitendinous, gracile, and patellar tendon grafts, and it is also possible to use Achilles and anterior tibial tendons. Their advantage, as mentioned before is that the complications of the donor site does not put an extra burden on the patient. Their further advantage technically is that their preparation does not increase the net operation time, with regards to narcosis and cut of blood supply. The deep freezed grafts have to be defrosed gradually and carefully, to preserve tissue structure. In case of tailoring it to the appropriate size, we have more freedom, choosing the length and thickness of the graft as needed.

We also have to state that, even with any professional surgical technique and careful graft selection, we cannot expect good results without appropriate rehabilitation. There exist numerous rehabilitation protocols that are very similar to each other.

Rehabilitation protocol

The rehabilitation protocol consists of well defined exercises that are built on each other. Even at the time of surgical planning, the patient has to be warned that the recovery will last for 6-12 months. The surgery should only be done if the patient is willing to take on the aftercare.

The rehabilitation protocol has to be tailored to the one's individual needs, and apart from his aptitude, the biology of graft incorporation as well. Ultimately, the important thing is that there is a two way information exchange between the patient and the physiotherapist, we reach our goal with cascading exercises, which in case of sports competitors means getting back to the previously achieved performance. In case of leisure sports people, the aim is to provide a good life quality with regards to the fact that leisure activities have an important sociopsychological aspect.
There is an agreement in the literature that depending on age and capabilities, the time of safe return is between 6-12 months.

Objective and subjective examination of patients with ACL replacement

Materials and methods

We examined injured who presented with ACL rupture at the Department of Traumatology and Hand Surgery and the Department of Sports Medicine between January 2013 and February 2015. In all cases we confirmed ACL rupture and instability with physical examination, and in some cases with imaging technique (MRI scan) as well. We excluded cases with accompanying or repeated injury from the study. Similarly, any concurrent previous lower limb injury with residual symptoms were grounds for refusal as well as neurological disorders.
In the study approved by the Regional and Departmental Research Ethic Committee, University of Pécs Clinical Center (file number: 4927), 70 injured (44 male, 26 female) participated, whose mean age was 39 (min. 16, max. 65), of which 58 injured (38 male, 20 female) composed the study group, who underwent anterior cruciate ligament plastics. In the study group, 34 people received hamstring, 11 patellar tendon (BTB) autograft and 13 people received allograft. The 12 injured (6 male, 6 female) who belonged to the control group, decided to refuse surgery after informed consent.
The surgical procedures were carried out in the Department of Traumatology and Hand Surgery at the University of Pécs by four experienced surgeons with transtibial tunnel technique, using Stryker Universal ACL Instrumentation System. The grafts were fixed proximally with Endobutton and distally with fast lock technique. We started physiotherapy immediately after the operation. After 3 weeks relief, physiotherapy was carried out at least three times a week for one hour in the department according to the protocol defined above. The group who underwent conservative therapy had the

treatment with the same protocol. The program contained sports specific exercises in the finishing stage of the rehabilitation.

The injured came for checkup 1,3,6,12 and 24 weeks after the surgery. We considered the therapy completed when the injured, following the principles of safe return to the field, could return to the activity they carried out before the knee injury. This, in agreement with the literature was attained between 6-12 months.

We performed the functional knee stability examination of the injured with Bretz DYNA 012 universal sports stabilometer. The measurement of thigh flexor and extensor muscle strength was done with TEDEA Huntleigh measurement cell and the attached Tenzi muscle force meter that included a computer program.

We analyzed our data with ANOVA and paired t-tests using SPSS 19 software. Significance level was set at $p<0,05$.

Stabilometry

The platform contains the sensors, with which we can measure the amplitude of effort force and the pressure point values of the arising forces of the person standing on it.

As a result of the measurements, the static and dynamic balance data appear numerically on the computer monitor. Our subjects performed all exercises standing on one knee, first on the healthy, than on the operated one.

Measurement of muscle strength

The second part of instrumental examinations consisted of muscular effort measurements of the thigh. We used a TEDEA Huntleigh type measurement cell and the attached computer program. During the test, the injured had to sit on a bench and raise a metal bar covered with a soft sponge with his leg (flexor insertion force) or press (extensor insertion force) first with the intact then with the operated leg, and we could read the extent of insertion force from the computer. We used this examination primarily to compare the operated and the healthy leg.

Subjective evaluation of patients after ACL replacement

Besides instrumental measurements, we used the 2000 IKDC SUBJECTIVE KNEE EVALUATION FORM and the Knee Injury and Osteoarthritis Outcome Score [KOOS] systems.
The aim of using the above subjective questionnaires was to evaluate the satisfaction level of the injured.

The results of the objective and subjective examination of patients after ACL replacement

Stabilometry

Static tests

In case of the static balance measurements carried out with the Bretz DYNA 012 universal sports stabilometer, significance was only found in one group, when tested with Open eye Romberg test (P=0.003), the ones who had the replacement with patellar tendon. There is an obvious tendency for the closed eye Romberg test in the conservatively treated group (P=0.050) (Table 1).
In the other study groups, we did not find substantial difference in terms of lower limb functional stability between the operated and healthy sides. This implies the restoration of proprioception and balance abilities during rehabilitation. Naturally, this is not possible witout proper muscle function.

Dynamic tests

The dynamic tests show a much more multifaceted picture.
Here, where task was the relocation of the center of gravity of the body, in the BTB group there was a significant difference for every test between the intact and the injured leg (Table 1). This, with the coexisting immaturity of proprioceptive reflex can imply a defect in the complex balance and movement regulation system. This, therefore, can strongly influence the results that showed a substantial difference between the two sides for the muscle force measurement of the extensor muscle group (see later). Therefore, it is possible that only the executive apparatus was injured and not the regulating mechanism.

Sport		Paired Differences					t	Sig. (2-ailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
					Lower	Upper		
konzervatív	R1	-1.00000	1.87083	.83666	-3.32294	1.32294	-1.195	.298
	R2	-5.60000	4.50555	2.01494	-11.19438	-.00562	-2.779	**.050**
	centrum	-.10600	.17097	.07646	-.31828	.10628	-1.386	.238
	cukor	-18.60000	35.47252	15.86380	-62.64496	25.44496	-1.172	.306
	egér	2.60000	6.58027	2.94279	-5.57049	10.77049	.884	.427
	színezés	1.00000	3.80789	1.70294	-3.72812	5.72812	.587	.589
	flexio	5.40000	5.81378	2.60000	-1.81876	12.61876	2.077	.106
	ext	6.80000	10.47378	4.68402	-6.20491	19.80491	1.452	.220
hamstring	R1	.02857	4.04741	.68414	-1.36176	1.41890	.042	.967
	R2	2.08571	7.23704	1.22328	-.40030	4.57173	1.705	.097
	centrum	-.00914	.18879	.03191	-.07400	.05571	-.287	.776
	cukor	-2.37143	18.92413	3.19876	-8.87209	4.12924	-.741	.464
	egér	1.31429	5.56127	.94003	-.59608	3.22465	1.398	.171
	színezés	.80000	7.32361	1.23792	-1.71575	3.31575	.646	.522
	flexio	9.31429	10.76697	1.81995	5.61570	13.01287	5.118	**.000**
	ext	4.05714	29.76075	5.03049	-6.16603	14.28032	.807	.426
btb	R1	-6.00000	2.82843	1.15470	-8.96825	-3.03175	-5.196	**.003**
	R2	-2.00000	2.52982	1.03280	-4.65489	.65489	-1.936	.111
	centrum	-.09000	.03225	.01317	-.12384	-.05616	-6.836	**.001**
	cukor	18.33333	13.70645	5.59563	3.94930	32.71737	3.276	**.022**
	egér	-4.00000	1.26491	.51640	-5.32744	-2.67256	-7.746	**.001**
	színezés	9.00000	3.40588	1.39044	5.42575	12.57425	6.473	**.001**
	flexio	.33333	1.36626	.55777	-1.10047	1.76714	.598	.576
	ext	69.16667	.40825	.16667	68.73824	69.59510	415.000	**.000**
allograft	R1	.00000	2.68328	1.09545	-2.81593	2.81593	.000	1.000
	R2	1.00000	4.64758	1.89737	-3.87734	5.87734	.527	.621
	centrum	.05000	.10991	.04487	-.06534	.16534	1.114	.316
	cukor	11.00000	8.53229	3.48329	2.04591	19.95409	3.158	**.025**
	egér	.00000	.89443	.36515	-.93864	.93864	.000	1.000
	színezés	1.33333	7.60701	3.10555	-6.64974	9.31641	.429	.686
	flexio	-2.16667	5.94699	2.42785	-8.40765	4.07431	-.892	.413
	ext	-2.66667	3.38625	1.38243	-6.22031	.88698	-1.929	.112

Table 1. Statistical analysis of stability indicators and the muscle force test measured by the stabilometer. Significant differences are in bold print. The stability measures of the patients treated with BTB are lower, which can be explained with the weakening of the thigh extensor that was also measurable. In case of the group replaced with hamstring, as expected, the thigh flexor muscle group was impaired noticeably. In both cases P<0.001.

Muscle force measurement

In case of the muscle force measurement, we got the expected results with TEDEA Huntleigh measurement cell and the attached Tenzi software.

In the hamstring group the flexion, in the BTB group the extension of the affected side was significantly impaired compared to the contralateral side. Both the conservatively

treated and the ones treated with allograft got good results, naturally, none of the muscle groups weakened to a significant degree (Table 1.).

Subjective examination results of patients with anterior cruciate ligament replacement

Examining the groups together, we did not find significant difference for any of the questions (pain, other symptoms, daily routine (ADL), sports and recreational activities (Sport/Rec) and quality of life (QOL), IKDC).

When, however we examined the groups in pairs, we got different results.

Comparing the BTB with the conservatively treated group, there was a substantial difference in favor of the operated group in terms of the quality of life (P=0.039).

When we compare the values of the group who received allograft with the conservatively treated group, they responded differently to the sports and recreational questions (P=0.025) and they differed in the IKDC assessment as well. This, together with the stabilometry data and the power force measurement could mean that despite the appropriate function and force, in case of the patients who refused surgery, less returned to their previous sports activity and evaluated their own condition less optimally.

Comparing the conservatively treated and the group who had allograft replacement, there is a major difference with regards to evaluating sports and recreation activity. Return to previous sports activity can be expected with a higher probability after operation, especially in case of replacement with an allograft.

None of the subjective values showed significant difference between the group that received allograft and the one with hamstring. In terms of assessing sports activity, however, there is a tendency, based on which the activity level of patients with hamstring graft is higher.

Lastly, on comparison of the BTB and the allograft group, the opinion of the two groups differs for the sports questions as well (P=0.046). There was also a significant difference in the responses of the IKDC questionnaire.

In view of the above, the statistical data revealed interesting differences that were only directed towards certain details between the different groups.

It is worth taking into consideration a simple diagram that shows the averaged points of the subjective assessment systems. On this, the values of each question answered

can be well seen. Even if there is not a significant difference between every two groups and regarding every question, the tendency is well represented.

It can be seen on the diagram, that the conservatively treated patients answered the most negatively to all questions practically. This, overriding the results of the objective measurements, should guide us to prefer surgery in the active age group. Following the diagram it is also apparent that the patients treated with allograft are the most satisfied with their condition. This is most prominent for the sports and IKDC score. The BTB and hamstring or in other words the patients who received autograft are in the mid-range (Figure 4.).

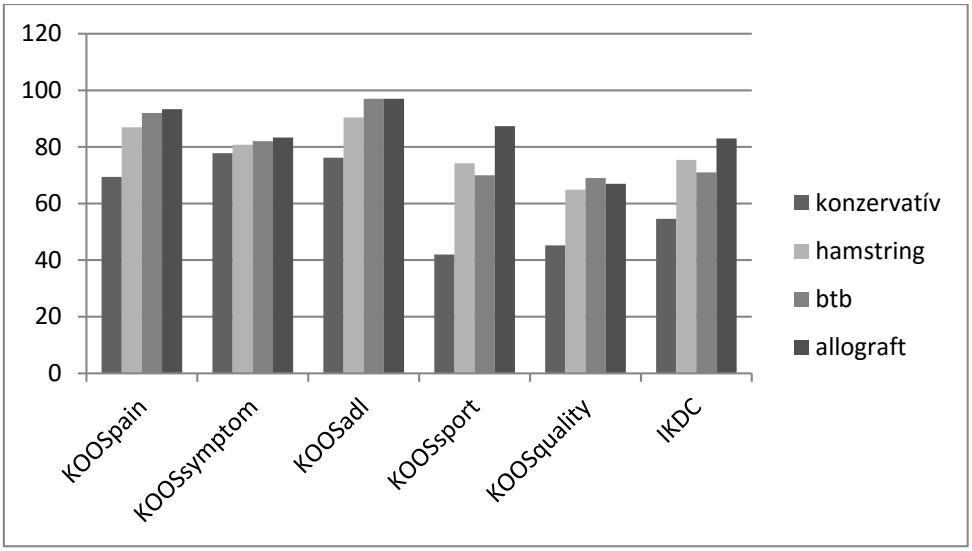

Figure 4. Average of subjective values. The tendency is obvious.

Preliminary summary

Summary of the results of objective and subjective tests

Summarizing the objective tests, it can be stated that they affirmed results close to our view. In my opinion, the alterations of functional stability are due to decreased muscle function. The objective and subjective results do not always correlate, the different groups, with very similar objective values assess their own situation very differently.

The results sometimes show parameters that that do not differ significantly, but in general, directly comparing the results of the different groups we can say that the conservatively treated group judged their own situation the worst, even though their objective parameters are not bad. The ones replaced with allograft are the most satisfied regarding pain as well as activity and lifestyle.

The effect of deep freeze on the structure of tendons used as allografts

Since the above examinations pointed out that using an allograft could be a good alternative in knee surgery, we were interested whether the time elapsed after donation has any importance in case of the tendons stored in -80 °C. In other words, does the collagen structure damage with time as a result of deep freeze, which could influence our surgical results?

In our clinical practice, we routinely use Achilles and patella allograft for the replacement of ACL. The aim of our study was to analyze the impact of the storage at -80 °C on the histological structure of Achilles and patellar tendons that we used for ACL replacement, and ultimately on the quality and incorporation of grafts. We expect from the results that the safe use of the grafts could be definable, ensuring good results.

Materials and methods

The allografts were removed in accordance with the 18/1998. (XII.27.) Ministry of Health regulation regarding the storage and certain patohistological examinations of organ and tissue transplantation. Following removal, we stored the tissues in the -80 °C freezer used for this purpose.

During the tissue removal that were carried out in accordance with the above mentioned legislation, we made sure that the donor site is not affected due to degeneration as a result of previous injury or underlying disease. During our study, we examined 4 patellar and 5 Achilles tendons. We took 0.5x0.5x2cm samples from the tissues and stored them in the tissue bank of the university. We carried out a histological examination and differential scanning calorimetry (DSC) from the fresh samples 1,2,6,12 and 24 weeks after the start of freezing.

Differential scanning calorimetry

Differential scanning calorimetry gives an opportunity to define the melting temperature of tissue structure, which is the native-denatured conversion point that takes place as a result of heat, and this characterizes the structural stability of tissues. The higher the temperature at which conversion takes place, the grater the tissue structure stability. We talk about scanning calorimetry when we control the temperature of the calorimeter according to a previously set program, and we measure the temperature of the processes parallel. The scanning calorimeters operate on a differential principle and

with temperature compensation, and are used in a wide temperature range. The differential scanning calorimetry (DSC) is the most suitable method for measuring the conversion temperature of solid materials. During the measurement process, we place a small amount of sample in an inert capsule, which is in most cases made of aluminum (Hastelloy vessel). We place the encapsulated sample in the sample holder of the DSC machine or on its measurement plate. The temperature range and the heating speed can be set with the attached regulatory control unit or with a computer.

We used a SETARAM Micro DSC-II calorimeter for this test. The measurements were carried out in a 0-100 °C temperature range in 850µl conventional Hastelloy vessel. We increased the temperature with 0.3 K/min. The weight of the samples ranged between 100-200mg.

We used RPMI-1640 solution as a reference. The sample and the reference solution was balanced with ±0.1 µg accuracy, therefore further correction was not needed in terms of heat capacity. We calculated calorimetric entalpy from the area under the curve of the heat absorption curve. We evaluated the results with OriginPro 7.5.

Histological evaluation

Parallel to the DSC tests, we also sent samples for conventional histological examination. For this, we placed the samples into 4% formaldehyde solution for 24 hours. Following fixation, we made longitudinal and cross sectional slices, and after paraffin embedding we made serial cuts and stained them with hematoxylin and eosin. We carried out the light microscopic examination with NIKON Eclipse 400 microscope.

Results

Reviewing the international literature, only a few used DSC to examine the allografts used for cruciate ligament replacement. The calorimetric test indicates structural changes of the molecules before they become visible. The aim of the investigation was to explore the structural changes of deep frozen tissues. Using calorimetry and classical histology we can predict the time until the tendon used is suitable for surgical use, thus contributing to choosing the optimal time of surgery.

The damage of the otherwise healthy collagen structure as a result of -80 C deep freezing can be histologically demonstrated. In case of Achilles tendon, this obvious damage occurs at the 6^{th} week. The separation and fragmentation of collagen fibers can be observed. The edges of collagen fibers appear blurred. The nuclei partly fade

and the eosinophilia decreases. Complete fragmentation is seen in some parts (Figure 5). In case of patellar tendon, the changes appear later, during the 24th week (Figure 6).

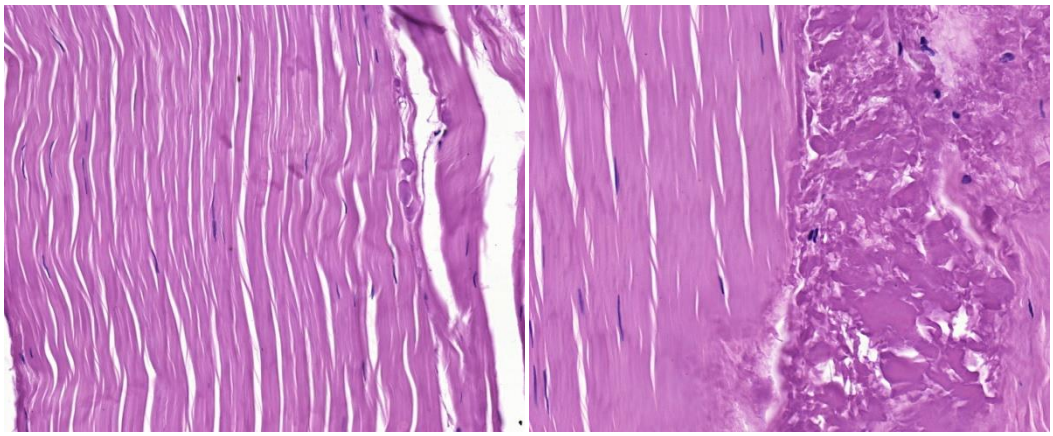

Figure 5. Left: fresh Achilles tendon with intact collagen structure. Right: the structural damages of the 6 week old deep frozen Achilles tendon, the fibers became fragmented. (Photograph: University of Pécs, Faculty of Medicine, Department of Pathology, Dr. László Kereskai).

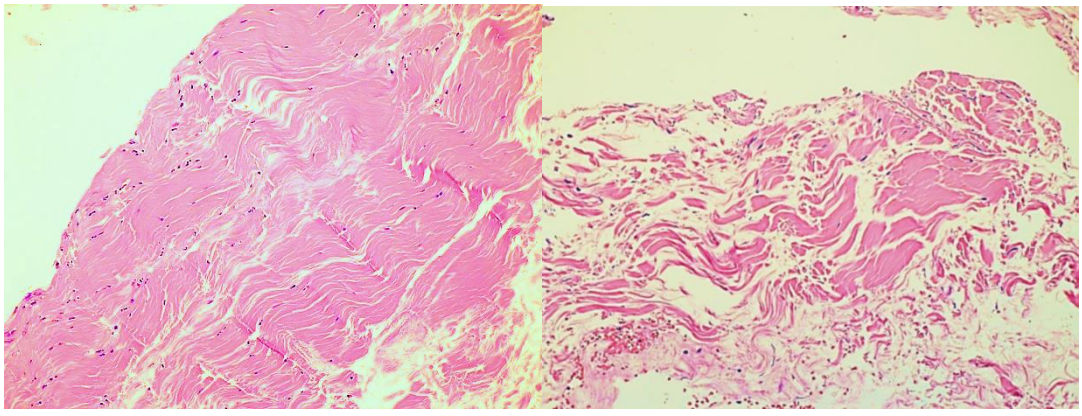

Figure 6. Left: section of fresh patellar tendon with H&E staining. Right: 24 week old deep frozen patellar tendon showing similar structural changes as the 6 week old Achilles tendon. (Photograph: University of Pécs, Faculty of Medicine, Department of Pathology, Dr. László Kereskai).

The DSC examination shows an obvious difference between the fresh and deep frozen samples. The thermal denaturation and calorimetric entalpy definitely shows the structural changes of the Achilles tendon with storage time. Table 2. and Figure 7. demonstrates the significant changes of thermal denaturation parameters that indicate fundamental changes of molecular structure in the Achilles tendon. In case of the Achilles tendon, the melting point, half width ($T_{1/2}$), temperature change (ΔT) and calorimetric entalpy (ΔH) change in the same direction until the 6th week. There is a sharp change observable at week 12th, which is probably due to water loss. The sudden change on the 24th week indicates the damage of the tendon structure. In case of the patellar tendon, the change of the melting point only starts at week 12th and becomes pronounced on week 24th. This means for us that surgical intervention can be carried out safely until week 24 (Table 2 and Figure 8). The results of the histological examination firmly support the results of calorimetry and they show the damage of collagen structures already on week 6 in case of Achilles tendon and on week 24 in case of patellar tendon.

Minta	**Termodinamikai paraméterek (átlag ± s.d.)**			
kezelési idő	**T_m/^oC**	**$T_{1/2}$/^oC**	**ΔT/^oC**	**ΔH/Jg^{-1}**
Achilles 0. hét	*63,8 ± 0,2*	*2,45 ± 0,1*	*18,77 ± 0,2*	*11,89 ± 0,58*
Achilles 1. hét	*64,6 ± 0,2*	*2,35 ± 0,1*	*22,99 ± 0,2*	*15,13 ± 0,73*
Achilles 3. hét	*64,21 ± 0,2*	*2,05 ± 0,1*	*23,0 ± 0,2*	*14,44 ± 0,61*
Achilles 6. hét	*64,02 ± 0,2*	*2,32 ± 0,1*	*18,26 ± 0,2*	*12,06 ± 0,49*
Achilles 12. hét	*64,55 ± 0,2*	*2,14 ± 0,1*	*22,14 ± 0,2*	*15,80 ± 0,78*
Achilles 24. hét	*64,49 ± 0,2*	*2.78 ± 0,1*	*21,26 ± 0,2*	*15,71 ± 0,77*
Patella 0. hét	64,07 ± 0,2	2,17 ± 0,1	23,11 ± 0,2	8,47 ± 0,37
Patella 1. hét	64,05 ± 0,2	2,62 ± 0,1	21,43 ± 0,2	12,29 ± 0,59
Patella 3. hét	64,27 ± 0,2	2,35 ± 0,1	22,53 ± 0,2	12,04 ± 0,58
Patella 6. hét	63,96 ± 0,2	3,26 ± 0,1	20,12 ± 0,2	13,57 ± 0,62
Patella 12. hét	64,77 ± 0,2	2,72 ± 0,1	22,13 ± 0,2	13,69 ± 0,63
Patella 24. hét	64,52 ± 0,2	2,25 ± 0,1	20,45 ± 0,2	12,10 ± 0,58

Table 2. The changes thermodynamic parameters of patellar and Achilles tendons with regards to the duration of deep freezing.

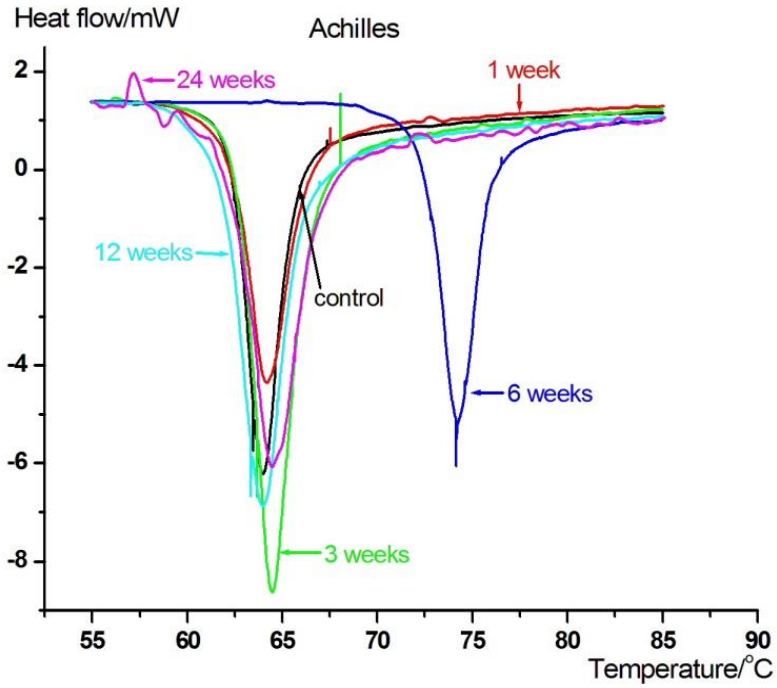

Figure 7. Calorimetric curve of the Achilles tendon. The changes of the curves from which entalpy is calculated is well visible.

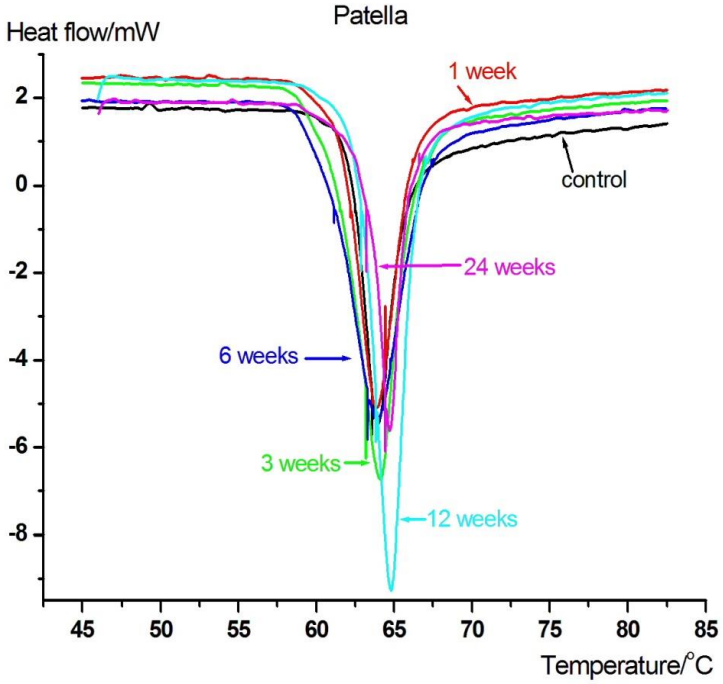

Figure 8. Calorimetric curve of patellar tendon. The entalpy, calculated from the area under the curve that changes as a function of time here as well, denotes macromolecular changes of collagen.

Taking the above results into consideration, we can state that if we aim to replace the cruciate ligament with Achilles tendon allograft, we have to do that within 6 weeks after the removal of the tissue. In case of patellar tendon, it can be demonstrated that the tissue damaging effect of the deep freezing starts much later, only during the 24th week. This also means that we have to choose the time of the replacement of the anterior cruciate ligament, if we want to use an allograft, adapted to the time of their removal, depending on the type of graft. Furthermore, we recommend that the results have to be taken into consideration not only for choosing the time of surgery but also that the tissue bank registration should contain the necessary data. Besides standardized surgical techniques and environment, we can optimize our intervention, improve our long term results with the recommended series of procedures in anterior cruciate ligament replacement taking the conclusions of the DSC examination into consideration during daily routine.

Summary

The anterior cruciate ligament is an important stabilisator of the knee. Its injury shows an increasing tendency in accordance with the changes in physical activity, leisure activity of the different social classes.

The treatment of anterior cruciate ligament rupture can be conservative, which is useful mainly for patients who are inactive or have underlying illnesses. In case of injured who are physically active, where there is demand for the return to previous lifestyle, there is surgical solution, which is nowadays routinely carried out in sports surgery.

The time of the operation depends on the local status of the knee, but it is also influenced by psychosocial factors. In case of early, within 3 week attendance, arthrofibrosis arises more often, whereas in case of late, beyond 1 year ligament plastics, the chance of further injuries increases and the early posttraumatic arthrosis development progresses as well.

According to my opinion, It is useful to give patients time to organize post-surgical rehabilitation and the lifestyle change. Moreover, with preconditioning, we can also improve their physical status facilitating post-surgical care. Therefore, we recommend to carry out the surgery in case of isolated cruciate ligament rupture at least 6 weeks after the injury.

A continuous change is observable regarding surgical technique and graft choice. The use of patellar tendon is reducing compared to the hamstring graft, the anatomical aim via an anteromedial port that respects anatomical proportions is now more widespread than the transtibial aim. After the transient increase in the number of two bundle replacement, nowadays the single bundle replacement has become more frequent again. Different reviews have been made evaluating long term results of anterior cruciate ligament replacement, but they have not found significant difference either regarding the method of aim, the number of bundles or the usage of auto versus allograft.

In case of choosing an autograft we do not have to account for disease transfer, their incorporation is safer and they are always at hand, but on the contrary, we have to take into consideration the complaints at the site of removal. This is more marked when using patellar tendon.

Using an allograft is more costly and has a greater administrational burden, but it does not load the patient with complications at the site of tendon removal.

In our study, we used a coherent surgical technique. After appropriate preparation, we performed the arthroscopic ligament reconstruction under quiescent tissue environment, with transtibial aiming, and one bundle technique. Fastening was also done with standard implants, femoral endobutton and tibial fast lock technique. The above mentioned uniform procedure gave us an opportunity to examine the effect of the employed graft on our results.

We performed our survey after completed rehabilitation, which, in accordance with international literature meant 6-12 months after the operation.

During our examination, we used stabilometer and muscle power measurement tools as objective methods. We assessed the satisfaction of our patients with IKDC and KOOS subjective questionnaires.

During objective measurements, in the conservatively treated patient group, the closed eye Romberg test showed significant difference between the healthy and the operated side. In case of using an allograft, relevant difference was shown for only one dynamic stability parameter. For patients where we used patellar tendon for anterior cruciate ligament replacement, the performance of the operated limb was worse both for static and dynamic parameters compared to the healthy side.

Regarding muscle force measurement, we got the expected result, the flexor force of patients who received hamstring graft was lower than the healthy side, whereas in case of patellar tendon the extensor apparatus was weaker to a demonstrable degree. In view of the above, we can state that based on the results of the objective measurement methods, we recommend hamstring graft plastics as the method of choice, but using the allograft could bring similar good results. The conservatively treated group also achieved good results, but the ones replaced with patellar tendon were not as successful in this comparison.

A question is whether this result shows in the subjective opinion of the patients.

Evaluating the IKDC and KOOS questionnaires, we came to the conclusion that despite good functional results, the ones who received conservative treatment are less satisfied with their condition. This also shows in that fact that less of them returned to their pre-injury sports activity. This might be due to the fact that they did not experience the post-surgical functional relapse, and pain, and instead compared the end result to the original situation. Maybe their psychological background ascertained that they decided not to undergo surgery, the fear and anxiety can imply that they did not take on the risk of returning to the previous sport activity. This however is a far-reaching conclusion and could be the subject of further research.

Summarizing the results of objective and subjective tests, we can state that we prefer surgical resolution for patients who live an active life, and the type of graft used requires weighing. In our opinion, the method of choice is the autologous hamstring graft, but allografts are good alternatives as well.

This brought the next question, what the limitations of using allografts are.

We demonstrated with differential scanning calorimetry and classical histological examination that the damage of the collagen structure can be demonstrated 6 weeks in case of Achilles tendon and 24 weeks in case of patellar tendon after the start of storage. We recommend taking these into consideration when using the different allografts for patient safety and good functional results.

Summary of novel results

In case of standard surgical situation and rehabilitation, and with good indication, we can achieve good results with any graft choice. Based on the indicators of the group who received allograft, it is recommended to choose this option for primary surgery as well.
The objective status does not necessarily correlate with the subjective satisfaction of patients. With similar objective parameters, the patients treated conservatively are less satisfied with their condition. In case of an active patient population we prefer surgery.

Examining different deep freezed allografts we can state that after a certain time has elapsed, their tissue structure damages. When using an allograft we have to pay attention to the length of deep freeze, in other words we can recommend to set an expiry date. We can tailor our registry to that.

The DSC test, after processing more samples seems to be suitable for revealing pathological structural changes at a macromolecular level before microscopic and macroscopic tissue destruction. Thus could forecast the usability of tendons as allografts.

Knowing the time of allograft donation, we can optimize the time of the planned cruciate ligament replacement, from which we can expect further improvements of surgical results and patient safety.

Acknowledgement

I thank Prof. Dr. Péter Than, the head of the doctoral program, and Dr. Norbert Wiegand and Prof. Dr. Dénes Lőrinczy, my supervisors for their hellp in my research and in writing my thesis.

I am grateful for Dr. László Vámhidy, my former boss who helped my work with conviction and affection.

I thank Dr. Ádám Horváth, for contributing to this work with his perseverance and interest.

I owe special thanks to Dr. Kata Gőcze, for persistently helping me with her knowledge and expertise.

I thank Dr. László Kereskai for the histological examinations.

I thank the physiotherapists at the Department of Traumatology and Hand Surgery, University of Pécs, Faculty of Medicine, for contributing to the recovery of my patients with their generous work. Special thanks goes to Judit Szakálas, who not only took over a great role in rehabilitation but also helped me with the measurements.