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Magnetic Resonance Imaging Evaluation of the Integration and Maturation of Semitendinosus-Gracilis Graft in Anterior Cruciate Ligament Reconstruction Using Autologous Platelet Concentrate

David Figueroa, M.D., Patricio Melean, M.D., Rafael Calvo, M.D., Alex Vaisman, M.D., Nicolás Zilleruelo, M.D., Francisco Figueroa, M.D., and Ignacio Villalón, M.D.

Purpose: To evaluate integration and maturation of semitendinosus-gracilis (STG) grafts in anterior cruciate ligament (ACL) reconstruction with magnetic resonance imaging (MRI) in patients who underwent ACL reconstruction with STG with and without autologous platelet concentrate (APC). **Methods:** A randomized single-blinded evaluator prospective study was performed in 2 consecutive series of patients who underwent reconstruction over a 14-month period: 30 with APC use (group A) and 20 as control subjects (group B). At 6 months, an MRI evaluation was performed, with observation of the graft's maturation and presence or absence of synovial fluid at the tunnel-graft interface. To facilitate interpretation, a scoring scale was designed to evaluate graft integration and maturation. **Results:** Regarding the presence of synovial fluid at the bone-graft interface, the test was negative in 86.84% of patients in group A and 94.74% in group B. A disorganized autograft signal pattern was found in 2.63% in group A and 5.26% in group B. Signal intensity was considered hypointense in 63.16% in group A and 42.11% in group B, isointense in 34.21% in group A and 52.63% in group B, and hyperintense in 0% in both groups. The final mean score was 4.45 points in group A and 4.2 points in group B ($P \geq .05$). Poor integration was found in 2.63% in group A and 5.26% in group B ($P = .214$). Good integration was found in 97.37% in group A and 94.74% in group B ($P = .784$). **Conclusions:** In our consecutive series of patients who underwent ACL reconstruction with STG grafts, 1 group with intraoperative APC use versus a control group, followed up by MRI at 6 months after reconstruction, we did not find any statistically significant benefit in the APC group in terms of integration assessment and graft maturation (ligamentization). **Level of Evidence:** Level III, case-control study.

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In the literature there is little information documenting the maturation and integration characteristics of semitendinosus-gracilis (STG) grafts with the bone tunnels in patients who have undergone anterior cruciate ligament (ACL) reconstruction with concomitant application of autologous platelet concentrate (APC).¹⁻⁵

At present, there is increasing interest in the use of APC in knee surgery, with the goal of improving tissue healing.¹⁻⁹ Activated platelets release the content of their granules, which enclose about 30 bioactive proteins (growth factors and cytokines). These cytokines attract macrophages, mesenchymal cells, and osteoblasts predominantly, removing necrotic tissue and stimulating tissue regeneration.

An argument can be made that APC may improve the mechanical properties of ACL grafts. However, no consensus exists. The current literature concludes that further clinical studies with greater levels of evidence are required to facilitate the understanding of the mechanism of action on different tissues when APC is used during ACL reconstruction.^{1,2,10}

The purpose of this study is to analyze integration and maturation of STG grafts with magnetic resonance imaging (MRI) in a group of patients who underwent ACL reconstruction and simultaneous application of APC, comparing them with a control group.

We hypothesized that the application of APC to the bone tunnels as well as the graft at the time of final fixation would lead to a more rapid integration with the bone tunnel and an enhanced ligamentization process.

METHODS

A prospective, randomized, single-blinded evaluator followed up 2 consecutive series of patients who underwent ACL reconstruction procedures with STG grafts over a period of 14 months.

With biostatistics, the sample's number was determined: assuming a 3% mean difference between both groups with and without APC use and an SD of 3%, with a confidence interval of 95% and statistical power of 80% (20% maximum β error), a minimum of 20 patients per group was required to achieve statistical significance at $P \leq .05$.

The total sample in our study was 50 patients having similar characteristics, who underwent reconstruction by the same surgical technique by the same group of surgeons, following the same postoperative rehabilitation protocol: 30 patients had concomitant application of APC (group A) and 20 patients without APC were used as control subjects (group B). Before the initiation of this work, the ethics committee of our institution approved this study.

The inclusion criteria were primary acute ACL rupture and patient's approval by signing an informed consent form regarding the procedure. The exclusion criteria were other types of reconstruction, other types of grafts, multiligament lesions, and inflammatory joint diseases. Postoperative complications were documented during follow-up.

APC use in ACL reconstruction with STG was performed in a simple aleatory manner: the possible benefits of APC use were discussed with each patient during the preoperative consultation in the sports medicine clinic; those who approved its use signed the

consent form, whereas those who declined APC use comprised the control group. Once we reached the appropriate number of cases in each group, statistical analysis was performed.

Concomitant to the surgical procedure, the APC was prepared by the technique provided by the manufacturer (Magellan system; Medtronic, Minneapolis, MN): we extracted 55 mL of the patient's blood and placed it in a test tube containing 5 mL of citrate. The sample was centrifuged at 3,200 rpm over a period of 15 minutes. After centrifugation, 3 zones were identified in the tube: the upper layer, containing platelet-poor plasma; the middle layer, containing platelet-rich plasma; and the bottom layer, containing erythrocytes. The middle layer (platelet-rich plasma or APC) was extracted and placed with an APC system syringe in the bone tunnels and in the graft once the reconstruction was finished, with all the intra-articular fluid instilled during arthroscopy having been previously removed. We applied 10 mL of APC under arthroscopy in both the tibial (3 mL) and femoral (3 mL) tunnels with the long needle syringe present in the Magellan system with the graft placed in its final position. The consistency of the APC when it was applied was almost gel like. After APC instillation into bone tunnels (6 mL including the femoral and tibial tunnels), the remaining 4 mL was directly applied in the intra-articular portion of the graft. In the MRI analysis of our series, graft ligamentization was evaluated by studying the imaging intensity of the intra-articular portion of the graft.

All cases analyzed in this study were reconstructed with hamstring tendons fixed in the femoral tunnel with the Transfix system (Arthrex, Naples, FL) and in the tibial tunnel with Delta absorbable screws (Arthrex) by 2 experienced sports medicine surgeons. The use of hamstring autografts with transtibial and single-bundle techniques and the procedures performed by the same surgical team allowed us to have standardized results, with a mean tibial and femoral tunnel length of 9 ± 1.3 mm (range, 7 to 11 mm).

Both groups of patients underwent the same accelerated rehabilitation protocol after ACL reconstruction, which consisted of immediate postoperative rest and continuous passive mobilization twice daily from the first postoperative day, in addition to ambulation with 2 crutches, isometric quadriceps exercises, and manual patellar mobilization (Table 1).

Six months after reconstruction, axial, coronal, and sagittal MRI sections with proton density-weighted, T1-weighted, and T2-weighted sequences were obtained to evaluate the characteristics of graft integra-

TABLE 1. Accelerated Rehabilitation Protocol After ACL Reconstruction

	Immediate POP	First Day POP	First Week POP	Third Week POP	Fourth Week POP	Second Month POP	Sixth-Eighth Month POP
Mobility	0°-90° in PCM	0° to 100°-120°	0°-120°	Complete	Complete	Complete	Complete
Walking	—	Assisted (2 crutches)	Assisted (2 crutches)	Assisted (1 crutch)	Free	Free	Free
Therapy	—	Patellar mobilization Quadriceps isometrics	Free ROM Patellar mobilization TENS Cryotherapy	Free ROM Patellar mobilization Quadriceps strengthening TENS Cryotherapy	Free ROM Patellar mobilization Quadriceps strengthening TENS Cryotherapy	—	—
Gym	—	—	—	—	Stationary bicycle Treadmill walk	Stationary bicycle Treadmill walk Dumbbell work	—
Sports	—	—	—	—	Swimming	Swimming Soft jogging	Return to sports

Abbreviations: POP, postoperatively; PCM, passive continuous motion; ROM, range of motion; TENS, transcutaneous electrical nerve stimulation.

tion into bone. A scoring scale was designed to facilitate evaluation of the grafts. The predominant signal intensity (>50% of graft surface) was used to define graft status when studied by MRI on the chosen plane, similar to all of the cases analyzed (Fig 1), in addition to the presence or absence of synovial fluid at the tunnel-graft interface (Fig 2). The 2 evaluated parameters have been validated in different previous studies.^{1,2,10} We defined scores in the range of 1 to 2 points as scarce integration and scores in the range of 3 to 5 points as adequate integration (Table 2). This scoring scheme has not been validated in the literature.

MRI scans were performed with a 1.5-T GE resonator (GE Medical Systems, Milwaukee, WI).

We documented the integration-measuring parameters in sagittal sections in a T2 sequence. The MRI scans were evaluated blindly by 2 radiology subspecialists in musculoskeletal pathology. To define the signal intensi-

ties of the grafts, they were compared with the intensities of the signals of the native semimembranosus muscle tendons in T2 sequences. The most useful images for comparison purposes were those corresponding to sagittal views. They were classified as hypointense (lower signal intensity than that of the semimembranosus muscle tendon), isointense (equal signal intensity to that of the semimembranosus muscle tendon), or hyperintense (higher signal intensity than that of the semimembranosus muscle tendon).

To determine the presence of synovial fluid in the tunnel-graft interface, fluid-sensitive sequences were used; T2 potentiated sequences were the most helpful for this purpose. If an area of higher signal intensity between the graft and the bone tunnel was observed, it was classified as positive for the presence of synovial fluid at the tunnel-graft interface. In the absence of this finding, the MRI scan was classified as negative

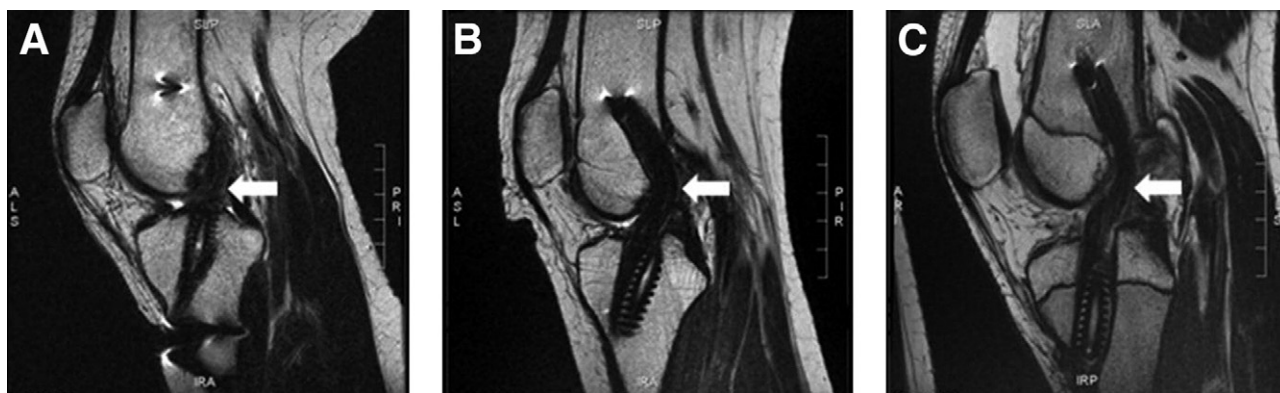


FIGURE 1. Sagittal MRI sections in T2 sequences 6 months postoperatively. (A) The arrow indicates the disorganized signal pattern in the graft. (B) The arrow indicates the hypointense signal pattern in the graft. (C) The arrow indicates the isointense signal pattern in the graft.

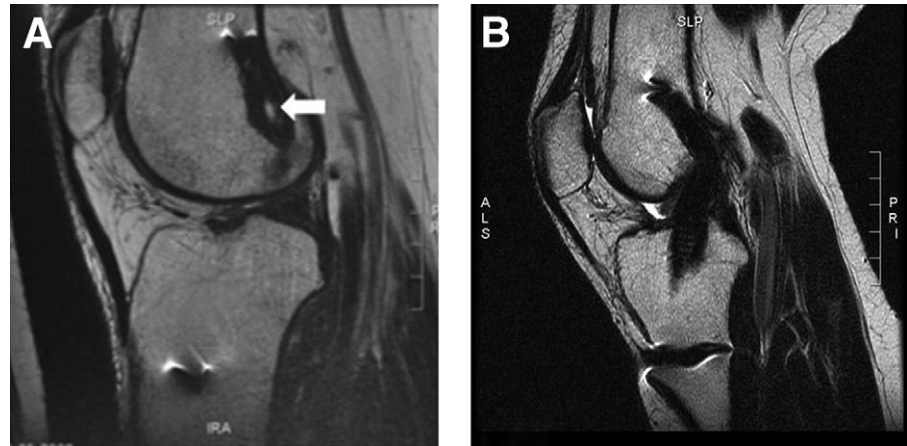


FIGURE 2. Sagittal MRI sections in T2 sequences 6 months postoperatively. (A) The arrow indicates a hyperintense white signal in the thickness of the bone tunnel, which documents the presence of synovial fluid at the bone tunnel-graft interface. (B) The absence of synovial fluid at the bone tunnel-graft interface is seen.

for synovial fluid at the bone tunnel-graft interface. Regarding synovial fluid presence at the bone-tunnel interface, MRI evaluations were performed in both the tibial and femoral tunnels. A positive finding was noted regardless of its location.

We defined our scoring scale in an arbitrary manner. Regarding graft signal intensity, 3 parameters could be reported: hyperintense, isointense, and hypointense. A score was assigned to each: 1, 2, and 3 points, respectively. Regarding synovial fluid presence, 2 possible findings could be reported: positive or negative. We assigned 1 point for positive and 2 points for negative synovial fluid at the graft-tunnel interface.

We also documented KT-1000 results (MEDmetric,

San Diego, CA) at 6 months' follow-up, at a mean of 6.4 months (range, 5 to 7 months) after reconstruction.

In the APC group (group A), there were 18 male patients and 12 female patients. The mean age was 26.8 years (range, 14 to 28 years), and MRI was done on average at 6.2 months (range, 5 to 7 months) after reconstruction. In the group without APC (group B), there were 15 male patients and 5 female patients. The mean age was 23.6 years (range, 13 to 35 years), and MRI was done on average 6.1 months (range, 6 to 7 months) after reconstruction. There were no significant differences between groups regarding gender, age, and time of MRI follow-up.

Statistical analysis of the continuous variables was done by comparing the mean values in the 2 groups. For categorical variables, we compared proportions, verifying the statistical significance through the χ^2 test (presence or absence of synovial fluid) and analysis of variance (graft intensity) for consecutive variables with $P < .05$. We did not find significant differences between groups considering gender and age ($P > .05$).

TABLE 2. Evaluated Parameters With MRI Use in ACL-Reconstructed Patients With and Without APC: Hamstring Graft Characterization Criteria

	Score (Points)
Integration: Synovial fluid at tunnel-graft interface (femoral or tibial)	
Positive	1
Negative	2
Ligamentization: Graft signal pattern (>50%)	
Hypointense	3
Isointense	2
Hyperintense	1
Characterization of graft	
Poor	2
Adequate	3-5

NOTE. Scores are assigned to each evaluated parameter (integration and ligamentization). The addition of both parameters allows us to classify the MRI characterization as poor or adequate.

RESULTS

In Table 3 we report the results of the MRI evaluation with respect to graft signal (>50% predominance), which was the first parameter studied. Isointense and hypointense signals were found in 97.37% of cases in group A and 94.74% in group B ($P = .784$). A disorganized fibrillar pattern was seen in 2.63% in group A and 5.26% in group B ($P = .214$). No grafts having hyperintense signals were seen in either of the groups.

After the evaluation, we scored the MRI findings according to the characteristics of the grafts (Table 2).

TABLE 3. Characterization of Graft in Percentages in APC Group and Control Group: MRI Evaluation

	APC Group	Control Group	P Value
Intensity			$P = .316$
Hypointense	63.16%	42.11%	
Isointense	34.21%	52.63%	
Hyperintense	—	—	
Disorganized	2.63%	5.26%	
Synovial fluid			$P = .720$
Positive	13.16%	5.26%	
Negative	86.84%	94.74%	

The mean scores after we evaluated the intensity characteristics of the graft were 2.58 ± 0.53 in group A and 2.32 ± 0.61 points in group B ($P = .316$).

The second parameter studied was the presence or absence of synovial fluid at the tendon-bone interface (Table 2). The corresponding mean scores were 1.9 points (range, 1 to 2 points) in group A and 1.96 points (range, 1 to 2 points) in group B ($P = .720$). These results correspond to the following percentages for synovial fluid at the interface: negative, 86.84% in group A and 94.74% in group B, and positive, 13.16% in group A and 5.26% in group B (Table 3).

The sum of the final mean values (both parameters analyzed) according to the imaging criteria was 4.45 points (range, 2 to 4 points) in group A and 4.2 points (range, 2 to 4 points) in group B ($P > .05$). We interpreted these results as showing good integration (3 to 5 points) in 97.37% of group A patients and 94.74% of group B patients ($P = .784$); we also found poor integration (1 to 2 points) in 2.63% and 5.26%, respectively ($P = .214$) (Table 4). It is important to note that this scoring scheme has not been validated in the literature.

At the last follow-up, 1 revision surgery was performed (including acute ruptures or chronic insufficiency) in group A and none in group B. We did not document secondary undesired effects or complications related to the use of intra-tunnel APC or other causes.

We obtained a mean measurement of 1.86 ± 1.81 mm on KT-1000 analysis of our series at 6.4 months (range, 5 to 7 months) after the reconstruction. Two cases presented with poor graft integration on the MRI study, with one case in each group (with and without APC). One of these cases (with APC) was classified as normal on KT-1000; the other case was classified as abnormal. The number of cases with poor graft integration was too small for statistical analysis.

DISCUSSION

Multiple factors contribute to the return to sports after ACL reconstruction with STG grafts. Regarding this point, integration of STG graft with the bone tunnel is a fundamental aspect to achieve in the post-operative stage.¹¹⁻¹³

The integration and ligamentization period (fibroblastic proliferation with production of type I collagen more inherent to ligaments than to tendons) consists of several phases with characteristic changes in each of them: in the initial phase, which begins between the first and second weeks after reconstruction, histologic analysis documents that there is central necrosis of the graft, together with hypocellularity without detectable revascularization. Then comes the proliferation phase, which presents maximum activity between the fourth and tenth weeks and is the most intense graft remodeling phase, presenting a precarious initial vascularization and cellular repopulation. Finally comes the ligamentization phase, where the graft's structural and biomechanical characteristics may become similar to those of an intact ACL, with the development of Sharpey fibers that show integration with the bone tunnels.¹⁴⁻¹⁸

Most studies have shown that this phase lasts at least 4 to 6 months. It has not been possible to define the end of this phase because some changes occur even years after the reconstruction; currently, this is a matter of discussion. These characteristic changes are documentable through an MRI study.¹⁹⁻²⁴

At present, there is increasing interest in the use of APC in knee surgery, with the goal of improving tissue healing.^{1,2,6,8} Activated platelets release the content of their granules, which enclose about 30 bioactive proteins (growth factors and cytokines). These cytokines attract macrophages, mesenchymal cells, and osteoblasts predominantly, removing necrotic tissue and stimulating tissue regeneration.

The APC is obtained by centrifuging plasma previously procured from patients and processed with special techniques and reagents, which provides a

TABLE 4. STG Graft MRI Characterization According to Scoring System We Designed to Evaluate Integration and Ligamentization on MRI in APC Group and Control Group: Final Scores of Graft-Tunnel Integration

Values	APC Group	Control Group	P Value
Intensity	2.58	2.32	.316
Synovial fluid	1.9	1.96	.720
Mean	4.45	4.2	> .05

platelet-rich plasma “super clot” that supplies growth factors to the zone where it is applied, hypothetically improving cell differentiation.²⁵⁻²⁹

It has been reported in the literature that the use of APC has been beneficial in spinal fusion²⁹ and, recently, in total knee arthroplasty, suggesting shorter hospital stays, less need for transfusions, and greater knee range of motion after 6 weeks, allegedly because of faster tissue healing around the prosthesis.^{6,7}

APC applicability in ACL reconstruction is still controversial, but there is evidence that at least the mechanical properties would improve. Further clinical studies with greater levels of evidence are required to facilitate the understanding of the mechanism of action on different tissues when APC is used during ACL reconstruction.^{1,2,10}

In the literature there is little information documenting the maturation and integration characteristics of STG grafts with the bone tunnels in patients who have undergone ACL reconstruction with concomitant application of APC.¹⁻⁵ With respect to the evaluation with MRI of soft-tissue graft incorporation after ACL reconstruction, Uchio et al.²² analyzed 64 patients with MRI scans and second-look arthroscopy 2 years after the procedure. They confirmed the biological attachment of the graft radiographically by the prior injection of contrast medium into the femoral bone tunnel. They concluded that a high-intensity MRI signal may indicate late fixation of the graft on the femoral tunnel, predicting possible post-reconstruction articular instability.

Similarly, using MRI, Murakami et al.²³ studied the changes in soft-tissue grafts after ACL reconstruction in the tibial bone tunnels. They reported that gradually within the tibial tunnel, a homogeneous low-intensity signal is produced 7 to 12 months after the reconstruction. They suggest that maturation of the tendon-tunnel interface is complete between 6 and 12 months after the procedure.

Recently, there has been increased use of APC during knee surgery, but little is known about the follow-up findings on MRI that we might observe after ACL reconstruction.

At present, only a few publications in the literature reporting on the use of APC in ACL reconstruction have analyzed the characteristics of the grafts in relation to their maturation and integration to the bone tunnels. Most of them conclude that further clinical studies with greater levels of evidence are required to describe the mechanism of action for APC after these procedures.

Ventura et al.¹⁰ analyzed a series of 20 patients

undergoing ACL reconstruction with STG, adding intra-tunnel growth factors delivered through the APC. At 6 months after the procedure, they performed computed tomography (CT) evaluation. Their results documented that the grafts in the group with APC were better structured and filled the tunnels better compared with the control group on CT scans. CT highlighted a significant difference ($P < .01$) between ACL density in the 2 groups and showed that ACL density was similar to the density of the posterior cruciate ligament in the growth factor-treated group. The authors concluded that in the group with APC, they saw faster “ligamentization” on CT scan analysis. This study has the weakness that the entire surface of the soft-tissue grafts was not evaluated by CT scan, because only the articular margin of the tunnel-graft interface was analyzed. MRI could offer a better understanding of their findings.

Radice et al.¹ published their experience with the use of APC in ACL reconstruction. They performed a prospective study of a series of 50 ACL reconstructions with intra-tunnel APC, adding coagulated APC across the thickness of the graft through a bioactive gelatin used for multiple purposes in medicine (Gelita; Gelita Group, Eberbach, Germany). They divided their series into group A, with APC, and group B (control). They obtained serial MRI scans at 3, 4, 5, 6, 7, 8, and 9 months after surgery in group A. In the control group they obtained MRI scans at 3, 4, 6, 7, 9, 10, 11, and 12 months. They recorded the characteristics of the graft in terms of the intensity seen on MRI and the presence of synovial fluid at the tunnel-graft interface. They concluded that the APC positively affects the remodeling process of the graft used, shortening the normal maturation time by 49.4%. This study has the weakness that despite performing a series of MRI studies to document the ligamentization characteristics, the study included a factor that could alter the interpretation of the graft’s characteristics in terms of its intensity, because the presence of the intra-graft Gelita could interfere with adequate interpretation of their findings.

In a recent study, Orrego et al.² described a series of 108 patients who underwent ACL reconstruction with STG grafts, dividing them into 4 groups: a control group, a group undergoing reconstruction with APC, a group in which they placed a bone plug in the thickness of the STG graft and the interface to the bone within the tunnels, and a group consisting of a combination of APC and intrasubstance bone plug at the graft-bone interface. The authors studied the patients with MRI at 3 and 6 months after the procedure. They

evaluated graft characteristics as to signal intensity and synovial fluid presence or absence at the bone-graft interface. Their results documented that the use of APC resulted in statistically significantly greater signal hypointensity compared with the control group, but this was not found when the APC group was compared with the groups that have a bone plug. They concluded that the APC improves the graft's maturation process when evaluated by MRI. These results were obtained through a statistical analysis that provides significance with respect to 4 groups; this point could alter the interpretation of the results obtained when a direct evaluation is made between 2 groups. Such a revision presents 4 different groups included in their study with multiple variables²; this could introduce bias in their results. The number of cases for each group was small, so it is possible that this might alter their results if analyzed with a greater number of cases. Our study analyzes this point in only 2 similar groups: one with APC use and one serving as control.

In our study we did not find significant differences between the 2 groups of patients (group A v group B) in relation to better integration or maturation of the graft evaluated by MRI studies at 6 months' follow-up.

In group A we found a trend toward a more hypointense signal of the graft evaluated by MRI; this could mean better ligamentization of the graft. Our results agree with what has been described in the literature, where we saw a tendency for maturation of the graft evaluated by MRI,^{1,2} showing greater hypointensity (better ligamentization) in the group treated with APC. However, when we evaluated the presence of synovial fluid at the tunnel-graft interface, we could not show any benefit in the group treated with APC, finding a greater number of cases positive for the presence of synovial fluid at the tunnel-graft interface in this group, which can therefore be interpreted as showing less integration to the bone tunnels. In addition, when comparing the mean imaging scores of the evaluated parameters (ligamentization and presence of synovial fluid) in both groups (group A v group B), we found greater scores in the group treated with APC, without statistical significance.

We cannot explain the reason for the increased number of positive cases when observing the presence of synovial fluid at the bone-graft interface in the APC-treated group. According to the discussed literature, this could indicate less integration of the graft to the bone.

Currently, it is not possible to assert this finding. This signal at the bone-tunnel interface could possibly

correlate to greater histologic activity, documented on MRI as a hyperintense signal. It is necessary to perform studies that correlate histologic and MRI findings to be able to determine this imaging characterization in an adequate way.

A weakness of our study is that the analysis of our patients was done with only 1 section at 1 given time. We do not know whether, in the long term, these differences will remain only as a trend or will become statistically significant. It is very important to continue with the proper follow-up of our series to report on the evolution of the patients from the standpoint of graft characterization by MRI, because it is possible that the effect of the APC is evident only during the first months after ACL reconstruction.

We studied MRI parameters described in the literature for the evaluation of graft integration and maturation at the bone-tunnel interface.^{1,2} A scoring system was designed to collaborate with the understanding of the imaging findings of each case analyzed. One important weakness of our study is that this scoring system has not been validated in the literature; therefore the imaging results presented in this study should be evaluated with caution.

APC was applied in the same manner in the femoral and tibial bone tunnels during arthroscopic viewing in all cases, with the surgeon placing the clot with the Magellan syringe system into the bone tunnels, with the graft placed in its final position, allowing them to bathe in the APC before fixation. This technique of APC placement is 1 limitation in this study, because we do not know the APC amount that stayed in the tunnel and we do not know how much APC bathed the graft. These points may produce variability in the amount of APC placed in each case. These could alter the MRI results obtained in our study.

CONCLUSIONS

In our consecutive series of patients who underwent ACL reconstruction with STG grafts, one group with intraoperative APC use versus a control group, followed up with MRI at 6 months after reconstruction, we did not find any statistically significant benefit in the APC group in terms of integration assessment and graft maturation (ligamentization).

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