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Vancomycin presoaking of hamstring autografts in ACL reconstruction is associated with higher MRI graft signal without influencing clinical outcome

Francisco Figueroa[1,2], David Figueroa[1], Rafael Calvo[1], Marilaura Nuñez[1], Gonzalo Serrano[1], Javier Barrera[1], Sven Putnis[3]

[1] Clinica Alemana-Universidad del Desarrollo, Santiago, Chile.

[2] Hospital Sotero del Rio, Santiago, Chile.

[3] Southmead Hospital, Bristol, UK

Corresponding author:

Francisco Figueroa

Address: Vitacura 5951, Santiago, Chile, 7650568

Phone: +56994304250

Email: franciscofigueroab@gmail.com

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Short running title: Vancomycin presoaking produces higher graft signal

Vancomycin presoaking of hamstring autografts in ACL reconstruction is associated with higher MRI graft signal without influencing clinical outcome

Abstract:

Purpose: To present the clinical and imaging results of a series of patients undergoing anterior cruciate ligament reconstruction (ACLR) with vancomycin presoaking of the hamstring autograft, compared to patients in the immediate period prior where no vancomycin was used.

Methods: A retrospective sequential series of patients with ACLR using either a non-vancomycin presoaking graft protocol (group 1, January 2013-October 2015) or vancomycin presoaking graft protocol (group 2, November 2015-December 2018). Lysholm and International Knee Documentation Committee (IKDC) scores were obtained at a minimum 24-month follow-up. Graft ruptures were recorded. Between 6-12 months follow-up, magnetic resonance imaging (MRI) was obtained to evaluate graft healing and integration.

Results: There were 102 patients (72% male), with 40 in group 1 (mean age 32.2 years) and 62 in group 2 (mean age 32.3). Five patients (13%) had a graft rupture in group 1 and six patients (10%) in group 2 (p=0.65). The median Lysholm score in group 1 was 95 [86-100] and 95 [90-100] in group 2 (p=0.37). The median IKDC score was 93 [82-99] in group 1 and 94 [86-99] in group 2 (p=0.22). MRI evaluation of integration found 87 patients (90%) had no synovial fluid at the tunnel-graft interface without differences between groups (p=0.24). Graft signal appearance found 45 patients hyperintense (46%), 45 (46%) isointense and 7 (7%)hypointens. Group 1 had a higher prevalence of hypointense grafts while group 2 had a higher prevalence of hyperintense and isointense grafts (p=0.003)

Conclusions: Vancomycin presoaking of hamstrings grafts increased the number of hyperintense and isointense grafts on MRI. Additionally, more hypointense grafts were noted when vancomycin was not used, suggesting the presence of more mature grafts in the non-vancomycin group.

Level of evidence: III, retrospective comparative study.

Introduction:

Knee septic arthritis after anterior cruciate ligament reconstruction (ACLR) is an uncommon but serious complication[1,2]. Even providing the best available treatment, there is a significantly increased risk of long-term joint dysfunction due to graft failure, and potential for severe articular cartilage damage when compared to an uneventful ACLR[3-6].

In an attempt to prevent this complication, Vertullo et al[7] in 2012 described a vancomycin presoaking technique for hamstring autografts, showing initial success in decreasing the infection rate after ACLR. After this research was published, an important number of articles using the same protocol have replicated these results (0% infection rate) including a variety of different graft types and concomitant procedures[8-17]. It also has demonstrated to be highly cost-effective[18].

Vancomycin has proven to be safe in terms of in vitro viability for the tenocytes, chondrocytes, and osteoblasts in the prescribed concentration[19-21]. In vitro data has also demonstrated no harmful effect on the strength and mechanical characteristics of graft[22-23]. However, the clinical and functional effects have not been studied in detail[10,12,24] and advanced imaging parameters such as magnetic resonance imaging (MRI) appearance are yet to be evaluated.

The aim of this retrospective review is to present the clinical and imaging results of a series of patients undergoing ACLR with vancomycin presoaking of the hamstring autograft, compared to patients in the immediate period prior where no vancomycin was used. The hypothesis is that vancomycin presoaking of the ACLR graft does not affect the clinical and functional outcome, or influence the graft appearance on MRI.

Methods:

We conducted a retrospective review in which we included consecutive patients who underwent arthroscopic primary ACLR with a hamstring autograft by 2 senior surgeons in a private hospital. During that period we initiated the presoaking of hamstring autografts with vancomycin. Institutional review board approval was obtained for this study.

Patients were divided into 2 sequential cohorts using consecutive periods: January 2013 to October 2015 (non-vancomycin protocol, group 1) and November 2015 to December 2018 (vancomycin protocol, group 2). All patients received a hamstring autograft. The exclusion criteria were ACL revisions, multiligament surgery, bilateral surgery, open concomitant procedures, less than 24-month clinical follow-up. Infected patients were also excluded because the objective of the study was to compare clinical outcomes between both groups in patients who did not develop septic arthritis.

The surgical technique consisted of hamstring autograft ACLR with a cortical button (Tight Rope, Arthrex, Naples, FL) on the femoral side and a soft tissue interference screw (Arthrex, Naples, FL) on the

tibial side. Hamstring graft preparation was planned using a standard 4-strand technique; grafts thinner than 8 mm were avoided by adopting a 5-strand technique[25]. The graft was presoaked in a vancomycin solution according to Vertullo et al[7], by wrapping the harvested and prepared hamstring graft in a surgical sponge previously soaked in a 5-mg/mL vancomycin solution (a dissolution of 500mg of vancomycin powder in 100ml of sterile saline solution). A surgical sponge is preferred over putting the graft directly in the solution, which can alter the graft diameter because of the fluid absorption[26]. The graft remained wrapped for at least 15 minutes while the arthroscopic stage of the reconstruction was performed. All patients also received standardized preoperative IV antibiotics consisting of a single 2-g dose of IV cefazolin.

Both groups of patients underwent the same accelerated rehabilitation protocol after ACLR, which consisted of immediate mobilization without restrictions from the first postoperative day, in addition to ambulation with weight bearing as tolerated with 2 crutches, isometric quadriceps exercises, and manual patellar mobilization. Patients were allowed stationary biking and swimming from the 4th postoperative week. After 12 weeks from the surgery, a comprehensive return to sport program was initiated with the objective of completing functional tests (isokinetic test, single hop) at the 6-month mark. If tests were successful, patients were allowed to return to sport without restrictions. If the tests were not successful, the patient continued the rehabilitation program until the tests were satisfactory.

As an institutional protocol, between 6-12 months after reconstruction, axial, coronal, and sagittal MRI sections with proton density–weighted, T1-weighted, and T2-weighted sequences were obtained to evaluate the appearance of the intra-articular reconstruction graft and appearance of the graft-bone tunnel interface using a previously published evaluation system[27] (Table 1). To define the signal intensities of the grafts, they were compared with the intensities of the signals of the native semimembranosus muscle tendons in T2 sequences. 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). The predominant signal intensity (>50% of graft surface) was used to define graft status[27]. MRI scans were performed with a 3-T GE resonator (GE Medical Systems, Milwaukee, WI). MRI scans were evaluated at two different times (separated by one week) by a radiology subspecialist in musculoskeletal pathology who was blinded to the patient group. AGFA IMPAX 6 software (Mortsel, Belgium) was used to evaluate the MRI

scans. There were no differences in time from surgery to MRI in both groups (Mean group 1: 243 days [SD 56.1], mean group 2: 233 days [SD 50.3]) (p=0.34). Lysholm knee scoring scale and International Knee Documentation Committee (IKDC) scores were obtained at final follow-up (February 2021). Graft ruptures were recorded.

Statistical analysis was performed using Chi-squared test and Fisher's exact test for means comparison. For qualitative variables, Student's-t and Mann-Whitney U test were used. A significant value was considered when $p<0.05$. MRI appearance was defined as the main outcome measure for the study. All data was processed in STATA version 16.0 (StataCorp, TX)

Results:

A total of 102 patients were included in the study (Figure 1): 73 males (72%) and 29 females (28%). Group 1 consisted of 40 patients (pre-vancomycin protocol) with a mean age of 32.3 years (range, 21-60) and a mean follow-up of 77.7 months (range, 64-95). Group 2 consisted of 62 patients (vancomycin protocol) with a mean age of 30.0 years (range, 18-54) and a mean follow-up of 37.5 months (range, 25-61). Both groups had a similar division of males and females (p=0.20), ages (p=0.22), and number of bundles in the graft (20 patients with a 5-strand graft in group 1, 26 patients with a 5-strand graft in group 2, p=0.42)

Regarding MRI parameters, 97 out of 102 MRIs were evaluated (because 5 patients had a re-rupture before the follow-up MRI was performed, 4 patients in group 1 [mean 8.1 months, range 6.8-10.9] and 1 patient [6.8 months] in group 2). When evaluating integration, 87 patients (90%) were considered to have absence of synovial fluid at the tunnel-graft interface without a statistically significant difference between groups (p=0.24) (Figure 2). Regarding graft appearance, 45 patients (46%) were considered to have a hyperintense graft, 45 patients (46%) an isointense graft and 7 patients (7%) were considered to have a hypointense graft. Group 1 had a higher prevalence of hypointese grafts while group 2 had a higher prevalence of hyperintense and isointense grafts (p=0.003) (Figure 3).

The median Lysholm score in group 1 was 95 points (IQR 86-100) and 95 points (IQR 90-100) in group 2 without a statistically significant difference (p=0.37). The median IKDC score was 93 points (IQR 82-99) in group 1 and 94 points (IQR 86-99) in group 2, again without statistically significant difference (p=0.22). Five patients (13%) presented with a graft rupture in group 1 and six patients (10%) in group 2

without statistically significant differences between groups (p=0.65) or between bundles in the grafts (p=0.27) In group 1 graft ruptures presented at a mean 10.3 months (range 6.8-13.4) and in group 2 presented at a mean 9.0 months (range 6.8-12.8) (p=0.29). All the ruptured grafts that had an MRI before rupture were evaluated noting hyperintense grafts without tunnel integration. No other complications, including infection, were noted in either group.

Considering MRI appearance as the main outcome measure for the study, a post-hoc power analysis was performed obtaining an 82% statistical power with the sample size available in the study.

Discussion:

This study has demonstrated that vancomycin presoaking of hamstrings grafts in a 5mg/ml solution increased the number of hyperintense and isointense grafts when evaluating an MRI taken between 6-12 months after the surgery. Additionally, more hypointese grafts were noted when vancomycin was not used, suggesting the presence of more mature grafts in the non-vancomycin group[28]. Integration of the graft at the bone tunnel interface saw no differences between groups. Patient reported outcome measures at a minimum follow-up of 24 months and the rate of graft rupture was not affected by vancomycin presoaking.

Recent literature has demonstrated a successful reduction in the infection rate after hamstring autograft ACLR when using a technique of vancomycin presoaking of the graft[29-32]. This technique has also been effective in reducing the infection rate in other types of grafts (patellar tendon, quadriceps tendon, allograft) and also in patients with concomitant ligament procedures or open procedures[29-31].

One of the major concerns about the vancomycin presoaking technique is the risk of potential damage to the graft cells. However, in vitro studies showed no deleterious consequences when it comes to its effect on tenocytes, when it is used in concentrations up to 5mg/ml (bovine tendons)[18], or up to 12.8 mg/ml (human patellar tendons)[20]. The study by Atherton et al[19] linked vancomycin use with a reduction in inflammatory proteins from treated tendon supernatants, suggesting that a reduction in these matrix protein and subsequent inflammatory cytokine release could point to a potential beneficial effect of vancomycin in generating a homeostatic environment and preserving graft integrity.

Previous studies indicate that the graft maturity and tendon bone healing level after ACLR could be evaluated by MRI[28]. In this study both parameters were evaluated on MRI between 6 and 12 months after

ACLR showing no differences in graft bone integration, but more mature intra-articular grafts where vancomycin was not used. How this can impact the reconstructions is unknown. Multiple studies have failed to demonstrate that the appearance of the graft on MRI at 12 months has influence on patient reported outcomes[33,34]. However, recently, Putnis et al[35] used image reconstructions to demonstrate that graft ruptures were associated with 12-month MRI appearances of high graft signal adjacent to and within the femoral tunnel aperture.

This study has demonstrated no effect of vancomycin use on clinical outcomes and there are previous studies demonstrating similar results. Bohu et al[10] in a case-control study found that the return to sport following ACLR was seen in professional or competitive athletes with good preoperative functional and psychological scores, no history of surgery, no medial meniscal injuries and no severe complications in the first year after surgery. Vancomycin use was not significant in the univariate and multivariate analyses. Perez Prieto et al[24] found that presoaking the graft in a 5mg/ml vancomycin solution in ACLR using hamstrings or patellar tendon grafts did not increase the risk of graft rupture or affect IKDC or Tegner scores. Offerhaus et al[12] showed no differences in the rate of postoperative arthrofibrosis, Tegner score or subjective outcome scores. Interestingly, the rate of graft ruptures was significantly decreased in their vancomycin group compared to the control group (3% failure versus 10%). The authors stated two possible theories for this finding; the simplest explanation being the longer follow-up in the control group and the second possible explanation the occurrence of low-grade-infections weakening the graft without any clinical signs of deep knee infections.

Limitations

It is relevant to note that as the patients included in the study were sequential, there are different follow-ups between groups. This is important as the group of patients with longer follow-up (non vancomycin) may have a higher chance of re-rupture because of the longer time between surgery and evaluation. However, there were no differences for graft rupture time between groups, and all the ruptures were between 6 and 13 months after reconstruction, with no later ruptures. The same issue could be applied for clinical scores follow-up. Further randomized studies with similar follow-ups between groups could eliminate this variable. Although sample size was adequate to obtain a statistical power larger than 80% for

MRI analysis and clinical scores outcomes, the study was underpowered to demonstrate significant differences in graft rupture. MRI evaluations were made by a single radiologist, which can affect reproducibility of the measurements. However, to ensure consistency, evaluations were made at two different times separated by one week and the internal consistency coefficient ensured excellent consistency. The retrospective design can introduce additional differences between groups and further prospective studies are required to confirm this study outcome, however, patients were of similar age and excluding the vancomycin presoaking technique, the surgical and rehabilitation protocols were identical for both groups. Finally, this study lacks of objective differential laxity measurements between groups (e.g. KT-1000 arthrometer) because we stopped measuring it due to the high variability between operators, as stated recently by Klasan et al[36].

Conclusions:

Vancomycin presoaking of hamstrings grafts increased the number of hyperintense and isointense grafts in MRI. Additionally, more hypointense grafts were noted when vancomycin was not used, suggesting the presence of more mature grafts in the non-vancomycin group. On the other hand, integration of the graft at the bone tunnel interface saw no differences between groups. Within this cohort, the vancomycin presoaking technique did not affect patient reported outcome measures or the rate of graft rupture at a minimum follow-up of 24 months.

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Table 1: Evaluated parameters in MRIs in ACL-reconstructed patients with and without vancomycin presoaking

Evaluated MRI parameters	Integration: Synovial fluid at tunnel-graft interface (femoral or tibial)	Ligamentization: Graft signal pattern (>50%)
	Presence	Hyperintense
	Non presence	Isointense
		Hypointense

Figure 1: Flowchart of patients included in the study.

Figure 2: Presence of Synovial fluid at the tunnel-graft interfance in groups with vancomycin and without vancomycin.

Figure 3: Graft signal pattern in groups with vancomycin and without vancomycin.