

Autograft Soaking in Vancomycin in Anterior Cruciate Ligament Reconstruction Alters Tendons Structure: an Histopathological Interpretation

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SUMMARY

Background. Anterior cruciate ligament rupture is a frequent injury in sports and infection after reconstruction is rare (0.14-1.8%). Intravenous antibiotic prophylaxis and soaking of autografts with vancomycin (tendons are soaked into a compress that contains 100ml of NaCl mixed with 500 mg of vancomycin for 10-15 min) have been applied in order to avoid infection after ACL reconstruction. The aim of this study was to determine if hamstrings soaking with vancomycin leads to histopathological changes than can produce functional impairment in patient's follow-up.

Methods. Assessment to the graft was made based on redundant tendon tissue from ACL reconstruction procedures of patients operated by the same surgeon. Tendons collected during surgery were used and surplus segments were submitted to histopathological study and elastin impregnation. Two samples from each tendon of eighteen patients were analyzed, with and without vancomycin impregnation.

Results. This study included 18 patients. The mean age at surgery was 29 years old, 83.3% were male and 16.7% female. There were no cases of documented septic arthritis. Our results showed a considerable reinforcement of elastin wavy configuration after soaking with vancomycin and apparent retraction of surrounding tissues.

Conclusions. Hamstrings autografts' structure is altered after vancomycin soaking. Histopathological analysis described in this study might produce a decrease in tendon resistance to tensile forces that was not correlated with an increased risk of rupture. Future studies are needed to clarify vancomycin effects on graft integrity.

KEY WORDS

Anterior cruciate ligament; vancomycin; antibiotic prophylaxis; knee infection; fibroblasts; elastin; collagen; hamstrings autografts.

INTRODUCTION

Anterior cruciate ligament (ACL) is one the most important stabilizing structures of the knee. Ruptures of ACL are common in sports, with an incidence > 200,000 per year at USA, most of them required surgical treatment (1). Septic arthritis after ACL reconstruction is a rare but potentially devastating complication, with an incidence rate between 0.14% and 1.8% (2, 3). The majority of these cases occurred between 2 and 6 weeks after surgery (4). Several risk factors are associated with septic arthritis such as diabetes, smoking,

hamstrings autografts, drains, increased operative time, open procedures, previous surgery at the same knee or concomitant procedures, use of tourniquets, among others (2, 3, 5, 6). The two overwhelmingly prevalent organisms isolated are coagulase-negative staphylococcus (62.5%) and *Staphylococcus aureus* (21.9%). Other commonly reported pathogens include *Propionibacterium acnes*, Enterobacteriae and Pseudomonas (4). It has been verified a higher incidence of infection with hamstrings tendon autografts compared with bone-patellar tendon-bone autografts and allografts, this

may be attributable to a more complex harvesting technique (more time needed *vs* bone-patellar tendon-bone harvesting time) and the use of intra-articular suture material (2, 5, 7, 8). A few authors correlated the high number of infections linked to staphylococcus to graft contamination with skin comensal bacteria, potentially during the harvesting phase. It has been shown a 14-23% contamination rate during ACL autograft harvesting and manipulation phases (9). Septic arthritis can lead to a poor outcome, chronic pain, arthrofibrosis, cartilage loss, degenerative arthritis and graft failure. Although graft removal can be avoided in most cases, several surgeries are needed as well as prolonged antibiotherapy, leading to significant socioeconomic costs. The apparent failure of antibiotic intravenous prophylaxis is thought to be due to the poor graft vascularization with levels of intravenous antibiotic below the minimum inhibitory concentration (MIC) capable to eliminate the most frequent pathogens (6). Vertullo *et al.* described in 2012 a novel surgical technique that besides the use of intravenous antibiotherapy associate presoaking of hamstrings grafts with vancomycin. In this technique the harvested hamstrings graft is wrapped in a surgical sponge that had been previously soaked in a solution that contains 500 mg of vancomycin powder in 100ml of sterile saline solution. Autografts are then kept in this solution for 10-15 min before surgical application. Local vancomycin has routinely been used among another's non-orthopaedic fields, such as vascular grafts and thoracic and spine surgery (4, 10). The choice of vancomycin is due to the fact that is a bactericid agent against skin comensal gram-positive bacteria; its pharmacokinetic characteristics make it appealing to local use: low allergenicity, heat stability, safety for local use, water soluble, large volume of distribution and low resistance rate (2, 4, 11). Some *in vitro* studies have shown some evidence of its low osteoclast and chondrocyte toxicity (12, 13). Recently, a study also reported that the use of vancomycin is cost-effective (6). Grayson *et al.* (12) have shown, *in vitro*, that the collagen fibres type I of the tendons, after soaking with vancomycin, can act as reservoirs for vancomycin, with the amount released and elution profile dependent on rinsing and tendon volume. Vancomycin elution was above the minimum inhibitory concentration for the most of bacteria. The elution of vancomycin is not sustained for long periods of time, in fact, the authors found that release occurs mainly between 10 and 60 minutes after impregnation. All the studies carried out until today (although most are level IV evidence) have shown that vancomycin is effective in reducing the rate of infection after ACL reconstruction (2, 3, 17, 18, 5, 8, 10-12, 14-16), reducing infection rates to 0.1% (1). The purpose of this study was to determine if hamstrings soaking with vancomycin leads to histopathological changes than might produce functional impairment in patient's *follow-up*.

MATERIALS AND METHODS

Material

We performed a prospective, case-control study. This study was approved by the Ethics Committee of our Department (favorable opinion on 22/04/2019, CE-003/2019). All patients included in this study signed an informed consent and are aware they will be part of a scientific publication. The study was conducted in accordance with the ethical standards established in the Declaration of Helsinki of 1946 (World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013 Nov 27;310(20):2191-4. Doi: 10.1001/jama.2013.281053). The datasets generated during the current study are available from the corresponding author on reasonable request. The inclusion criteria in this study were: patients with acute-ACL ruptures submitted to ACL reconstruction with hamstring autografts by 2 senior surgeons from our Hospital, who applied the same surgical protocol. For graft fixation, the method used was the same in all patients – a suture button-based femoral cortical suspension (EndoButton fixation technique) and an interferential screw for tibial fixation. Patients with multi-ligamentous knee injury and chronic injuries were excluded. All patients who met these criteria from May 2019 to May 2020 were included, corresponding to 18 patients (**table I**). The mean age at surgery was 29 years old, 15 patients were male (83.3%), and 3 patients were female gender (16.7%). All patients received antibiotic prophylaxis with 2 g of intravenous cefazolin, 30 min before starting of surgery (before tourniquet inflation) or 600 mg of clindamycin if there was a knowledge history of allergy to penicillin.

Table I. Study's population.

Case	Gender	Age at time of surgery
1	M	42
2	M	28
3	M	16
4	M	43
5	M	34
6	M	36
7	M	46
8	M	43
9	F	38
10	M	24
11	M	29
12	F	20

Case	Gender	Age at time of surgery
13	M	19
14	M	24
15	M	48
16	F	38
17	M	28
18	M	27

Methods

The first step of all surgeries was semitendinosus (ST) harvesting. Autologous grafts from semitendinosus tendons were collected. Simultaneously a solution with 500 mg of vancomycin powder in 100 ml of NaCl had been prepared. A surgical sponge was soaked in this solution. After harvesting and preparation of the hamstrings, they were wrapped in a surgical sponge soaked with a solution of 500 mg of vancomycin diluted in 100 ml of a saline solution and left there for 15 min, before being surgical applied.

Two samples were taken from each tendon:

- sample 1: obtained after graft harvesting (without vancomycin);
- sample 2: obtained after impregnation with vancomycin.
- Both samples were collected from redundant tendon tissue that was not use for graft preparation.

Afterwards, the collected samples of step 2, were formalin-fixed and formalin-embedded. Beyond Hematoxylin-Eosin, elastin fibres argentic impregnation was also realized. Histopathological study was developed at Institute of Anatomical and Molecular Pathology, Faculty of Medicine, University of Coimbra.

Optical interpretation was developed by comparing the two samples of each tendon.

A bibliographic review on the topic was carried out with consultation of the Medline database and taking into account the degree of clinical evidence.

RESULTS

Patient follow-up

There were 18 patients who met the inclusion criteria. All patients followed the same postoperative rehabilitation protocol. They were examined at 2 weeks, 6 weeks, 3 months, 6 months and 1 year of post-op. All patients completed at least 6-month follow-up.

There were no cases (0%) of documented septic arthritis or graft failure. Diagnosis of septic arthritis was based on clinical examination (fever, knee effusion, knee inflammatory

signs), laboratory parameters (CRP and ESR) and bacterial cultures of synovial fluid of the knee joint.

There were no reported allergies or secondary effects to surgical procedure due to the preparation with vancomycin soaking. No patient had a recorded allergy or an adverse event.

Histopathological interpretation

Firstly, the structural changes in the autologous hamstring tendons of our group were evaluated by Hematoxylin and Eosin staining of tissue sections. Microscopy images showed an organized collagen network with tenocytes arranged in rows parallel to collagen fibres, with delicate wavy configuration, in hamstrings without vancomycin soaking (**figures 1, 2, 3**). All tendons with vancomycin soaking showed an increased wavy configuration. In addition, the alignment, or orientation, of the collagen fibres was altered and disrupted in the vancomycin soaking group (**figures 4, 5, 6**).

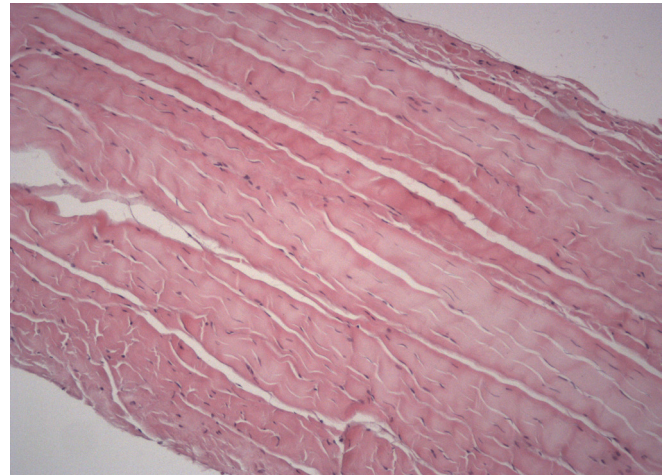


Figure 1. Section of hamstrings tendons stained by hematoxylin and eosin without vancomycin soaking - case 16.

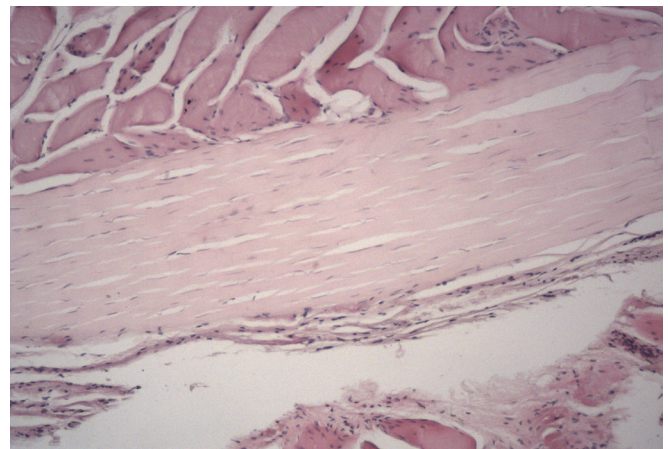


Figure 2. Section of hamstrings tendons stained by hematoxylin and eosin without vancomycin soaking - case 12.

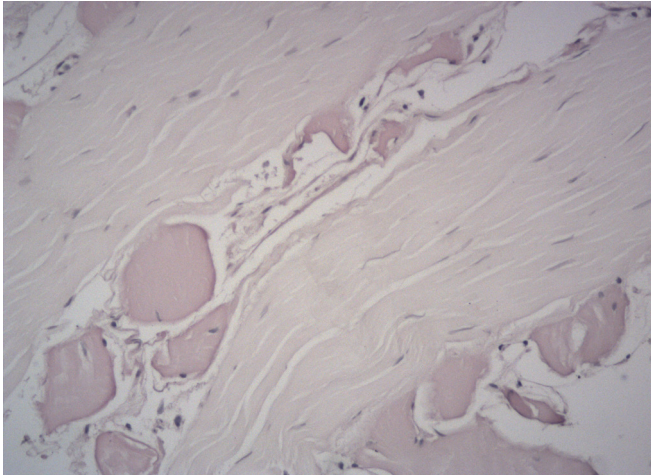


Figure 3. Section of hamstrings tendons stained by hematoxylin and eosin without vancomycin soaking - case 11.

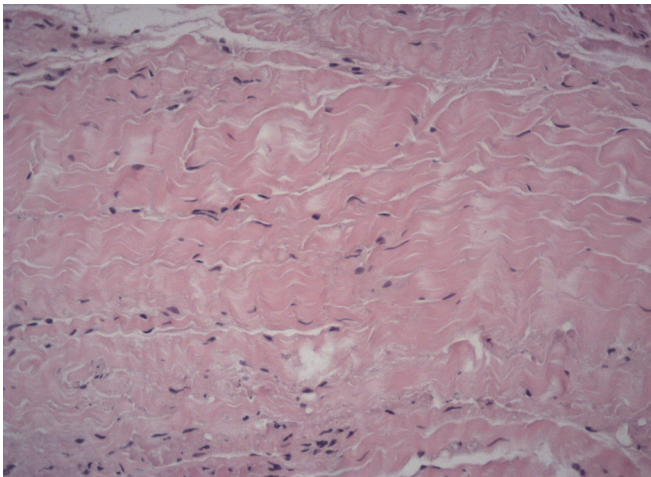


Figure 4. Section of hamstrings tendons stained by hematoxylin and eosin with vancomycin soaking - case 14.

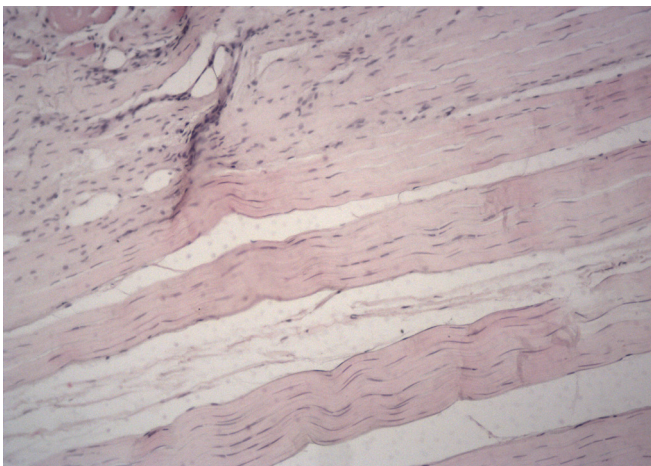


Figure 5. Section of hamstrings tendons stained by hematoxylin and eosin with vancomycin soaking - case 12.

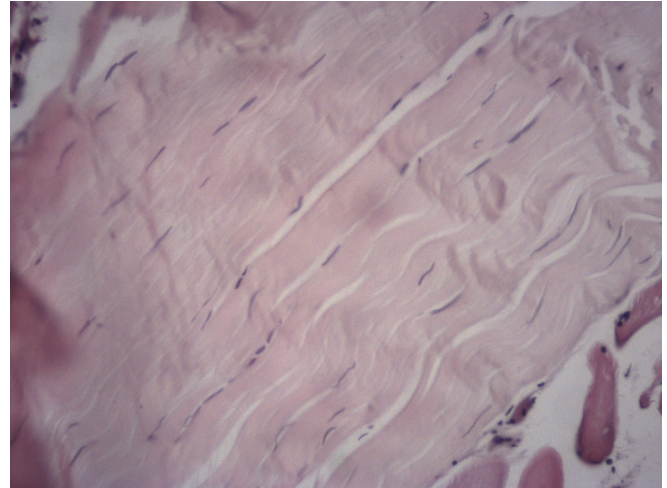


Figure 6.Section of hamstrings tendons stained by hematoxylin and eosin with vancomycin soaking - case 11.

Elastin impregnation

Elastic fibres are not clearly evident in traditional Hematoxylin and Eosin (H & E) stains of tissue sections. The applied technical procedures indicated that elastic fibres were densely distributed around tenocytes and between fascicles. Elastic fibres were longitudinally oriented along the tendon. **Figures 7, 8 and 9** show the elastic fibres orientation without vancomycin impregnation. The observation showed considerable reinforcement of elastin wavy configuration after vancomycin soaking and apparent retraction of surrounding tissues (**figures 10, 11, 12**).

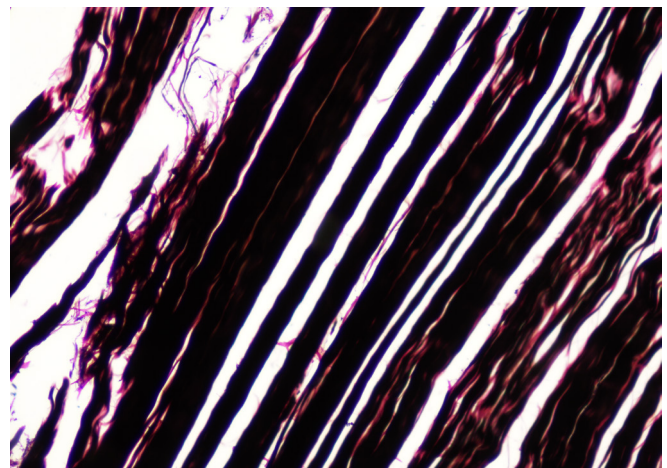


Figure 7. Elastin impregnation. Section of hamstrings tendons without vancomycin soaking - case 6.

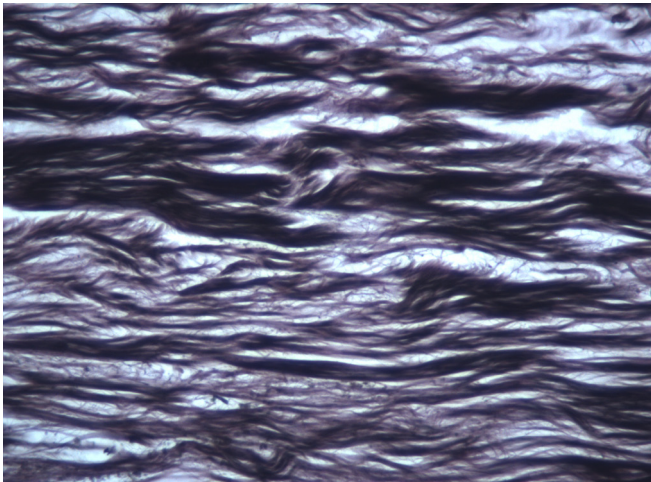


Figure 8. Elastin impregnation. Section of hamstrings tendons without vancomycin soaking - case 3.

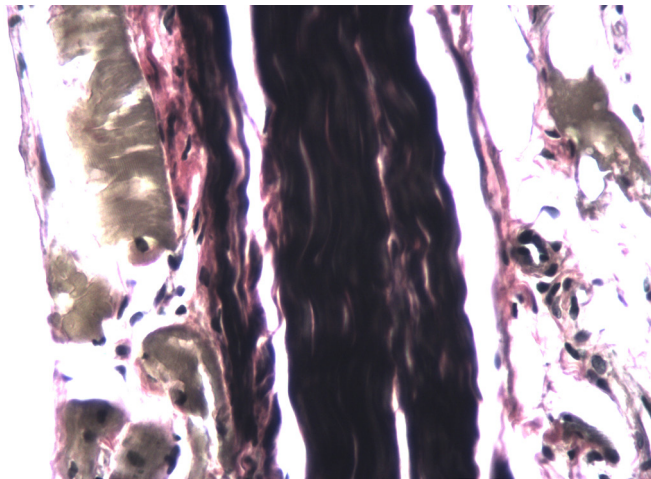


Figure 9. Elastin impregnation. Section of hamstrings tendons without vancomycin soaking - case 9.

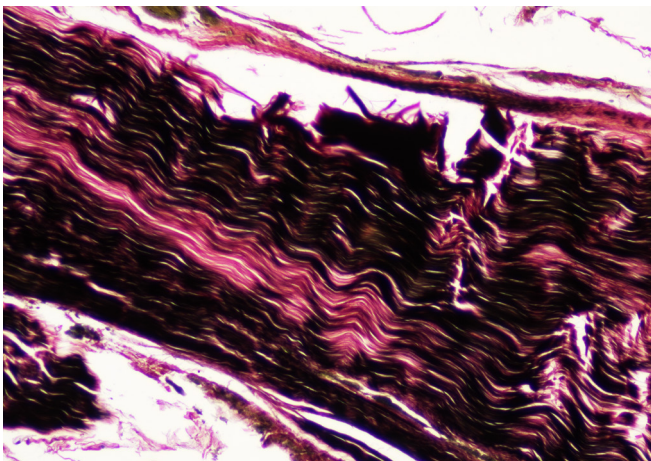


Figure 10. Elastin impregnation. Section of hamstrings tendons with vancomycin soaking - case 1.

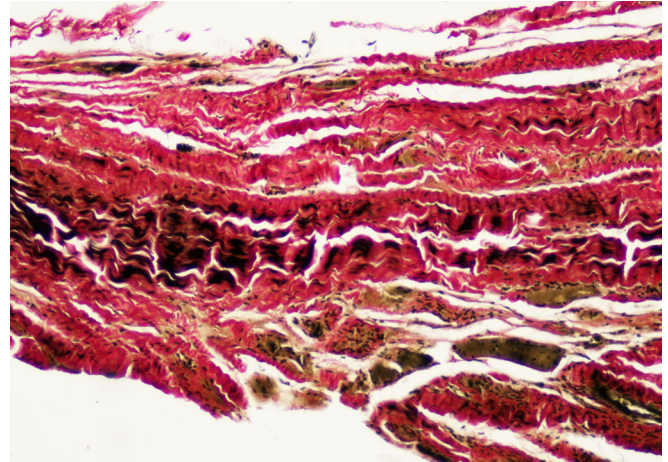


Figure 11. Elastin impregnation. Section of hamstrings tendons with vancomycin soaking - case 14.

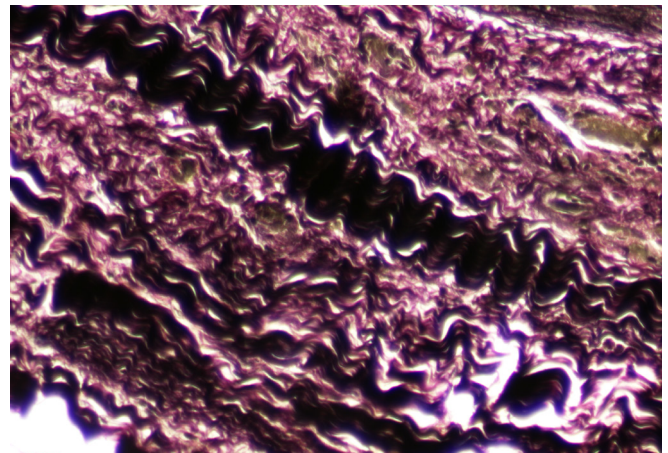


Figure 12. Elastin impregnation. Section of hamstrings tendons with vancomycin soaking - case 3.

DISCUSSION

To our knowledge, this is the first study to present the histopathological features of hamstring autografts soaking with vancomycin solution. The hamstring autografts used for ACL reconstruction showed marked histopathological changes.

Infection after ACL reconstruction is a rare but catastrophic complication. Major concerns following ACL-reconstruction infection are graft survival and long-term graft function. Although graft removal is rarely needed, long-term antibiotics and long hospital stays are usually necessary, with high socioeconomic costs associated. Several techniques have been studied to decrease infection rate. Recently, many studies showed that graft pre-soaking with vancomycin decrease infection rate. Vancomycin's choice lies in its pharmacokinetic proprieties such as low allergenicity, heat stability, safety for local use and large volume of distribution

(15). The use of vancomycin-soaked grafts was associated with a 10-fold reduction in the rate of postoperative infection after ACLR (16). For more than 10 years orthopedic surgeons have been using this technique but the literature surrounding this technique is very limited. Adverse effects, local and disseminated toxicity to cells and biomechanical properties are almost unknown. Every single study showed with no doubt that the use of vancomycin reduces the risk of infection after ACL-reconstruction from low to minimal (2, 5, 10, 14, 15), but there are some concerns about vancomycin safety, allergic reactions and potential antibiotic resistance development.

Recently, Braun *et al.* reported that even small concentrations of vancomycin have a negative effect on proliferation and functionalization of various cell types (19). They studied the cytotoxicity of vancomycin on osteoblasts, endothelial cells, fibroblasts and skeletal muscle cells. The most sensitive cells were the human skeletal muscle cells.

There are no guidelines for the local administration of antibiotics. Especially in local therapies the concentration of antibiotics is crucial to surrounding tissues viability. It's known that tendons function as reservoirs of vancomycin (12). The amount of vancomycin released, and elution profile depend of tendon volume, soak solution concentration and on rising. It's unknown if the amount of vancomycin that stayed in autografts could damage tendon's structure. Liu *et al.* (20) concluded that continuous vancomycin exposure (48 h) has a significant cytotoxic effect on proliferating osteoblast and myoblasts at concentrations greater than or equal to 1 mg/cm², and for fibroblasts at concentrations greater than or equal to 3 mg/cm².

With this study we pretended to know histological effects of vancomycin in surrounding tissues, such as collagen and elastic fibres. The most important finding of this study was the change of tendon structure after vancomycin soaking. After vancomycin soaking tendons showed an increased wavy configuration, that fact was confirmed by histopathological interpretation in all cases. Several studies showed that in the resting state, the collagen fibres and fibrils of a tendon show a wavy configuration which appears already under the light microscope. This configuration disappears if the tendon is stretched slightly corresponding to a straightening of the collagen fibres. When the tensile force is released, the tendon resumes its normal wavy appearance (21). In this studied both of the samples were in resting state, and after vancomycin soaking an increased waving configuration was seen.

Recently investigators have been interested in a hypothetical mechanical role for elastic fibres in tendons. The mechanical stability of the tendinous collagen is the most important factor for the mechanical strength of a tendon. The function of elastic fibres is not entirely clear, but they may contribute to the

recovery of the wavy configuration of the collagen fibres after tendinous stretch (21). Elastic fibres are present in low quantities in tendon (1-2%), where they are located both within fascicles near tenocytes and more broadly in the interfascicular matrix. Elastin is an important load-bearing tissue in the bodies of vertebrates, used specifically in tissues requiring high fatigue resistance or energy storage, as a result of its highly elastic mechanical behavior and resilience. Recent studies suggested that elastin had an important role in tendon healing process, elastin and fibrillin-1 are upregulated in torn tendons (22).

A recent study reported a slightly higher rate of postoperative arthrofibrosis in the vancomycin group (6), this study of 2018 was the first study to provide data regarding possible side effects of vancomycin such as graft failure, arthrofibrosis and subjective outcome measurements. Bohu *et al.* (17) affirmed that vancomycin presoaking does not increase the risk of ACL recurrent tears and that return to sport is not altered. Other studies had the same conclusions (18, 23). The biomechanical impact of vancomycin presoaking has not been well investigated until now and the majority of the studies are in animals (24). Ekdahl *et al.* (25) reported that only 8% of Swedish surgeons included in their study used vancomycin presoaking. The lack of guidelines on this subject should make us alert; studies in this area are urgently needed. It's questionable if the available data recommend the universal application of this technique for all patients undergoing ACL-reconstruction or if it only should be done in special conditions such as revisions surgeries, type of graft used (increased risk if HT autograft or allograft are used), patient risk factors (such as diabetes, smoking) and procedure-associated factors (such as increased operative time, concomitant open surgical procedures, long tourniquet inflation time, use of a drain).

New techniques describe the use of biodegradable polymeric materials, such as fast-resorbable hydrogel coating (Defensive Antibacterial Coating [DAC], Novagenit Srl, Mezzolombardo, Italy) that releases antibiotics in the knee joint after orthopedics procedures. A recent study (26) reported the use of DAC and vancomycin in ACL deficient patients and the authors said that is safe and effective. This method can be an option when tendons cannot be used – for example, in total knee joint arthroplasty. In ACL reconstruction the tendons function as reservoirs of vancomycin and there is no need to use these carriers.

Different limitations can be attributed to this study. First of all, the limited number of patients, our samples were taken from patients who underwent arthroscopic ACL reconstruction. The ideal sample should be the entire tendon, which could be done with allografts or animal samples. Another possible limitation of this study is the use of only semitendinosus autografts. We used only two staining methods (Haematoxylin and Eosin; Elastic impregnation). We

used these techniques because they were cost-effective, widely available and easy interpreted. Further techniques as electron microscopy weren't used, so we possible underestimate tendon abnormalities. It's difficult to have randomized controlled trials in this area because the number of required patients would be extremely high due to rare incidence of infections after ACL-reconstruction.

It's a fact that the structure of the tendons has been changed, but there's still an outstanding question remaining; if histopathological observed changes are result of duration of immersion in vancomycin or due to the concentration used. It's also unknown whether these structural changes are correlated with tendons biomechanics modifications and consequently an increased rupture rate after ACL reconstruction. Further studies are required to clarify vancomycin effects on graft integrity.

CONCLUSIONS

Vancomycin as bactericidal agent against skin commensal bacteria has shown evidence of low toxicity on chondrocytes and osteoclasts with releasing rate into tissues over time.

Although infection after ACLR is a rare complication, it can have catastrophic effects, leading to graft failure and joint disfunction.

Semitendinosus tendon structure is altered after vancomycin soaking. This study showed an increased wavy configura-

tion in all patients of vancomycin group. In addition, the alignment, or orientation, of the collagen fibres was altered and disrupted in the vancomycin soaking group. Future studies are needed to clarify vancomycin effects on graft integrity beyond infection concern. These findings raise concern about ACL preserved function after surgery and the actual vancomycin application.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

CQ, JPO, FF: study conceptualization and design. CQ, JPO, PM, AI, FF: data collection. CQ, JPO: analysis and interpretation of results. CQ: draft manuscript preparation.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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