Article published online: 2020-01-09

Intraoperative graft decontamination during ACL reconstruction surgery*

Descontaminação transoperatória de enxerto durante cirurgia de reconstrução do LCA

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Rev Bras Ortop 2020;55(4):410–414.

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Abstract

Objective To evaluate different decontaminants for tendon grafts, proposing an antiseptic protocol for contaminated grafts.

Methods A total of 25 patients were tissue donors for the study. Each participant donated a 2.5-cm tendon sample, which was divided into 5 fragments with 5 mm each during anterior cruciate ligament (ACL) reconstruction surgery. The collected material was divided into 5 groups, totaling 125 samples. In total, four fragments of each patient were placed on the operating room floor for one minute for contamination, simulating the fall of the graft on the floor during surgery. The other fragment was immediately placed in a sterile container (group 1). One of the contaminated fragments was placed in the sterile container without being previously immersed in decontaminating solution (group 2). The remaining fragments were immersed for ten minutes in decontaminating solution: 0.5% chlorhexidine (group 3), 0.9% saline (group 4) and 0.55% ortho-phthalaldehyde (group 5), and, after this time, they were individually placed in a sterile container. The samples from the 5 groups were submitted to microbiological examination.

Results Bacteria were detected in 26% of the total samples in the microbiological tests, and in group 1 there was no growth of microorganisms. In group 2, bacterial growth was observed in 16 samples. Considering the evaluation of test groups 3, 4 and 5, the percentage of decontamination was higher than the growth of microorganisms in the respective cultures.

Conclusion The protocol suggested by the study showed that intraoperative graft decontamination is possible.

Keywords
- autograft
- anterior cruciate ligament
- decontamination

Resumo

Objetivo Avaliar diferentes descontaminantes para enxertos de tendões, propondo um protocolo de antissepsia para o enxerto contaminado.

* *Study developed at Hospital Orthomed Center, Uberlândia, MG, Brazil.*

received
March 28, 2019
accepted
July 31, 2019

DOI https://doi.org/10.1055/s-0039-1700830.
ISSN 0102-3616.

Métodos Um total de 25 pacientes foram doadores de tecido para o estudo. Cada participante doou uma amostra de 2,5 cm de tendão, a qual foi dividida em 5 fragmentos de 5 mm durante cirurgia de reconstrução do ligamento cruzado anterior (LCA). O material coletado foi dividido em 5 grupos, totalizando 125 amostras. Ao todo, quatro fragmentos de cada paciente foram colocados sobre o piso da sala cirúrgica, durante um minuto, para contaminação, simulando a queda do enxerto no chão durante o ato operatório. O outro fragmento foi, imediatamente, colocado em um recipiente esterilizado (grupo 1). Um dos fragmentos contaminados foi colocado no recipiente esterilizado sem ser previamente imerso em solução descontaminante (grupo 2). Os demais fragmentos foram imersos, por dez minutos, em solução descontaminante: clorexidina 0,5% (grupo 3), soro fisiológico 0,9% (grupo 4) e ortoftaldeído 0,55% (grupo 5), e, após esse tempo, foram colocados individualmente em um recipiente esterilizado. As amostras dos 5 grupos foram submetidas a exame microbiológico.

Resultados Houve detecção de bactérias em 26% do total de amostras nos testes microbiológicos, sendo que no grupo 1 não houve crescimento de micro-organismos. No grupo 2, observou-se crescimento bacteriano em 16 amostras. Avaliando-se os grupos de teste 3, 4 e 5, o percentual de descontaminação foi superior ao crescimento de micro-organismos nas respectivas culturas.

Conclusão O protocolo sugerido pelo estudo mostrou que é possível a descontaminação transoperatória do enxerto.

Palavras-chave
- autoenxerto
- ligamento cruzado anterior
- descontaminação

Introduction

Anterior cruciate ligament (ACL) reconstruction is a frequent surgical procedure, with good results.[1] Autologous grafts are the preferred ones; however homologous grafts (tissue bank) have often been used.[2–4]

Accidental contamination during intraoperative graft management, including falls to the operating room floor, may occur.[2,5] Accidental fall is a major cause of graft contamination during surgery.[4,6]

The implantation of a contaminated graft may lead to septic arthritis, one of the most feared joint complications, with an incidence between 0.6% and 1.8% in ACL reconstruction surgeries.[4,7]

The absence of well-established decontamination protocols increases the incidence of postoperative septic arthritis in ACL reconstruction, decreasing the functional success rate of the joint.[8–10]

There is no consensus on the best decontaminant to be used during intraoperative graft contamination. The most commonly used products are 2%chlorhexidine and 0.9% saline.[10–15]

The present study may be important to establish an intraoperative graft decontamination protocol, reducing the incidence of postoperative septic arthritis.

Materials and Methods

The methods for graft decontamination during ACL reconstruction surgery were evaluated.

The present study was approved by the Ethics in Research Committee of our institution, under the Certificate of Presentation for Ethical Appreciation number 60893316.0.0000.5704, opinion number 1.962.752, on March 13, 2017.

A total of 25 patients, male and female, aged between 18 and 53 years, with indication for ACL reconstruction with autologous flexor tendon graft, participated in the study. Samples that did not match the size or that were altered prior to microbiological testing were discarded. Each patient donated the necessary exceeding length of the tendon for the graft. This excess part of the graft was transformed into 5 samples with 5 mm each. Each 5-mm sample was part of 1 of the 5 test groups, totaling 125 samples.

Group 1: uncontaminated samples.
Group 2: contaminated samples not immersed in decontamination agents.
Group 3: sample immersed in 0.5% chlorhexidine.
Group 4: sample immersed in 0.9% saline.
Group 5: sample immersed in 0.55% ortho-phthalaldehyde.

The samples from groups 2, 3, 4 and 5 were placed on the operating room floor, where they rested for 1 minute. The samples from group 2 were not immersed in decontamination agents, and were placed in a sterile container. The samples from groups 3, 4 and 5 were kept immersed in containers of 100 ml of their respective decontaminants for 10 minutes. After this time, they were placed in sterile containers. All samples were sent for microbiological examinations (Gram and culture).

After collecting the data, each of the decontamination methods was assessed and quantified by statistical analysis using the Statistica (TIBCO Software Inc., Palo Alto, CA, US).

The Chi-squared test is one of the most used tests in biomedical research, being applied to the data measured in nominal or ordinal scales.

Results

Descriptive analysis of samples

The tissue samples were grouped into 25 packages from different patients. The patients were not separated by gender or other variables. The graft sample donated by the patient was divided into five parts, corresponding to the number of test groups. The samples from group 1 were not contaminated. The samples from the other four groups were placed on the operating room floor so that they could be contaminated. The immersions were performed in three containers with different solutions (test group), so that the graft fragments were decontaminated. The results of the microbiological examinations of the 125 samples were analyzed.

Bacteria were detected in 26% of the total samples in the clinical trials, regardless of the test group, as shown by ►**Figure 1**.

►**Figure 2** shows the presence of contamination in the samples, after the evaluation of the 25 packages, regardless of the test group. "P" corresponds to positive Gram clinical tests.

Test groups 1 and 2 are control groups. Group 1 showed negative clinical tests in all packages. Group 2 showed positive clinical trials in 16 packages. Considering test group 2, some propositions are made. The occurrence of contamination evidenced by the clinical test was of 64% of the sample. As it is a control group, we state that in 64% of the cases of exposure to contamination, the tissue is effectively contaminated. We treated this value as a reference for some of the following tests.

When evaluating the test groups with decontaminating solutions, we observed that the occurrence of decontamination was higher than the presence of contamination, as shown by ►**Figure 3**.

Statistical Analysis

The statistical analysis was performed with the aid of the Statistica and BioEstat 5.0 software, and applying the Chi-squared test for the measured data in nominal or ordinal scales, and to evaluate the existence of trends related to the solution used in the test group and to the decontamination of the tissue.

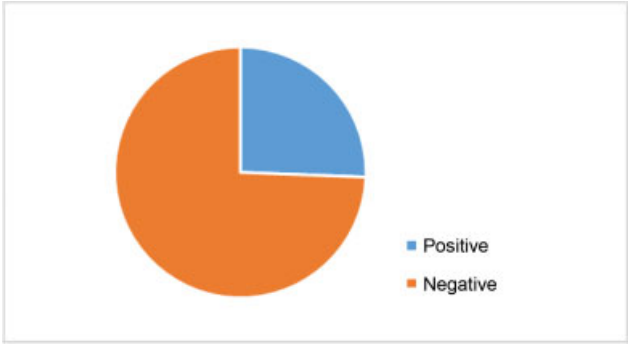

Fig. 1 Gram clinical test result for the total of samples.

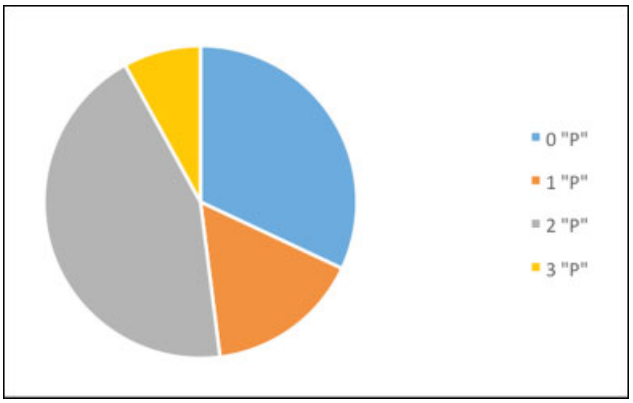

Fig. 2 Quantity distribution of positive results in the 25 analyzed packages.

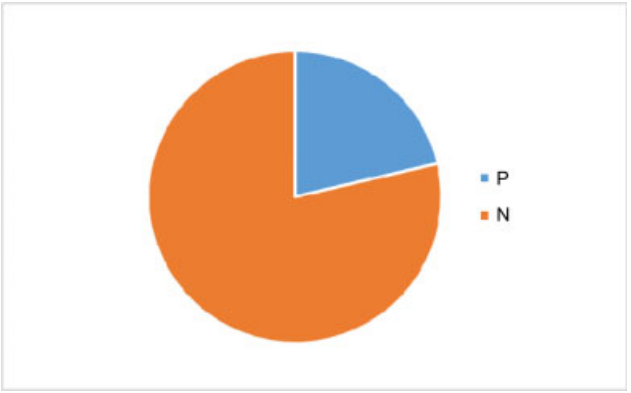

Fig. 3 Quantity distribution of negative and positive results regarding test groups 3, 4 and 5.

►**Table 1** presents the data entered to perform the test described.

The result showed that there is no association of events (contamination or not) with the investigated test groups. The test is not significant ($p = 0.7719$), indicating no tendency for the low positive value of A (2.0000). Thus, it is not possible to reject the null hypothesis (H_0: there is no trend of test group regarding the largest number of contaminated tissues). Considering success as "N" for each test group, the test result would be the same, only with a negative A value of the same magnitude.

The Chi-squared test for equal proportions was applied in the event of tissue decontamination by test group, relative to the proportion observed in test group 2 (control), in order to

Table 1 Positive (P) and negative (N) data for the clinical test by test group

	Group 1	Group 2	Group 3	Group 4	Group 5
P	0	16	0	14	2
N	25	9	25	11	23

evaluate the adherence of the values found in the sample in relation to a control reference. This is the most widely-used non-parametric proof in the medical sciences. The prevalence of contamination in a population is 0.64. Thus, the hypotheses are formulated:

H_0: the proportion of negative results agrees with that expected in the control group: $p_1 = p_2$;

H_1: the proportion of negative results is not as expected in the control group (aseptic solution effective for N < P): $p_1 \neq p_2$.

Test group 3: the corrected Chi-squared is significant ($p < 0.0001$), evidencing that the observed values do not agree with those expected in the control group. Therefore, the null hypothesis is rejected, and the alternative is accepted. The existing difference classifies test group 3 as an effective antisepsis solution for tissue decontamination.

Test group 4: the corrected Chi-squared is not significant ($p = 0.6892$), evidencing that the observed values agree with those expected in the control group. The existing difference is, therefore, sample variation.

Test group 5: the corrected Chi-squared is significant ($p < 0.0001$), evidencing that the observed values do not agree with those expected in the control group. Therefore, the null hypothesis is rejected, and the alternative is accepted. The existing difference classifies test group 5 as an effective antisepsis solution for tissue decontamination.

Applying the odds ratio technique, which is a test for proportions arranged in a 2×2 contingency table, we obtained for *test group 5* that the probability of decontamination is about 20 times higher than that of the control group, with $p < 0.0001$. On the other hand, for *test group 4*, the probability of decontamination is not significant (nonexistent), with $p = 0.7728$.

Discussion

Although all surgical procedures have a potential risk of contamination,[16,17] the present study showed that by strictly following the proposed protocol, the decontamination rate is safe.

The results obtained showed a decontamination rate of 100% with 0.5% chlorhexidine.

This shows that the proper and safe strategy is the adoption of a decontamination protocol with 0.5% chlorhexidine.

This protocol proved to be efficient in graft decontamination, providing safety to the surgeon.

The prevalence of septic arthritis after ACL reconstruction is of 0.1% to 0.9%.[16,17] One in four sports medicine orthopedic surgeons may experience ACL graft contamination during their careers, according to a research by Izquierdo et al.[6]

The risk of contamination after graft fall is high. About 60% of tissue samples that fall to the ground have positive bacterial cultures.[18] Contamination can also occur without dropping the graft, as shown at a level II study,[19] in which 12% of ACL autografts were contaminated during preparation for reconstruction.[19]

In the case-control study by Abdel-Aziz et al.[20] infection was controlled in all cases without graft sacrifice. However, the clinical results of the infection group were lower than the results of those without infection.

If the graft falls to the ground, a correct protocol and sterilization agent are required for decontamination, and they should be readily available if graft preservation is considered. Graft sterilization and preservation result in lower patient morbidity, and are a more attractive option for contaminated grafts if an efficient protocol is used.[1]

According to Badran and Moemen,[21] the hamstring graft contamination rate after a fall to the ground was of 50%. In the present study, the occurrence of contamination evidenced by the clinical test was of 64%.

Clinical research indicates that about 75% of surgeons in graft contamination situations use some tissue decontamination technique and then implant it. As for the remaining surgeons, 18% use contralateral limb autografts, and 7% use allografts.[19]

Clinical studies indicate that, after graft contamination, most surgeons place the contaminated graft in chlorhexidine solution for periods between 90 seconds and 30 minutes so they can be decontaminated. The supplementary methods reported also included pulsatile lavage or mechanical tissue agitation.[1] In our research, the immersion time in decontaminant solution was of 10 minutes, and no supplementary method was used.

In the present study, we obtained a rate of 100% of decontamination with 0.5% chlorhexidine, 92% decontamination with 0.55% ortho-phthalaldehyde, and the 0.9% saline solution did not show a significant decontamination rate ($p = 0.6892$). In 90 contaminated samples that were sterilized with chlorhexidine, decontamination was successful in 98%.[1]

The present analysis did not evaluate the biomechanical effects of the solutions used in grafts. However, it is important to consider the effect of sterilization solutions on the mechanical properties of the grafts. Chlorhexidine belongs to the bisbiguanide antiseptic class; it has documented cytotoxicity against fibroblasts, and negatively affects cell proliferation. The effect on wound healing is controversial.[22]

A decontamination protocol with 3 L of 2% chlorhexidine irrigation has been published. The study showed no change in maximal graft load for failure, final stress failure or stiffness.[23]

Data regarding this topic in the literature corroborate the results of the present study, and enable the formulation of a tendon graft decontamination protocol in ACL reconstruction surgery. Thus, the decontamination protocol can be proposed as follows: once the graft has fallen to the ground, an auxiliary table with sterile fields and sterile containers must be set up. The graft should be deposited in enough 0.5% chlorhexidine solution for full graft immersion, and it should be kept there for 10 minutes. After this period, the graft can be implanted in the recipient site, following the surgical technique in use.

Conclusion

In the present study, we concluded that it is possible to promote tissue graft decontamination in ACL surgeries during the intraoperative period, provided the technique used follows the indicated protocol and use of an effective sterilizing agent. The present study confirms its working hypothesis, as well as

the hypothesis of other studies already published, which claim that the use of autograft is safe, even if the tissue has been contaminated by a fall to the ground.

Conflict of Interests
The authors have none to declare.

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