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No association between positive intraoperative allograft cultures and infection rates after reconstructive knee ligament surgery

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ABSTRACT

Background: Several reports of severe infections associated with allograft tissue in knee reconstructive surgery have led many surgeons to consider routine intraoperative culture of allograft tissue before implantation. Thus, the purpose of this study was to determine the prevalence of positive soft tissue allograft cultures in reconstructive knee surgery, and evaluate its association with surgical site infection.
Methods: Retrospective study of 202 patients who underwent knee reconstructive ligament surgeries, including revisions, between January 2013 and July 2017. Intraoperative culture results were obtained and the report of a surgical site infection during follow-up was recorded. Patients without cultures were excluded. A priori power analysis was performed. The association between positive culture results and development of surgical site infection was evaluated using Fisher's Exact test ($P < 0.05$).
Results: A total of 300 allografts were implanted in 202 patients. Mean average follow-up was 32.9 ± 12.5 (range 13 to 57.9) months. Sixteen patients had positive intraoperative allograft cultures (7.9%). The most frequently isolated organism was *Bacillus* species (six cultures); none of these patients presented with clinical signs of infection. Nine patients developed surgical site infections and were treated with oral antibiotics, and one patient developed septic arthritis that required surgical debridement of the implanted graft; all of these patients had a negative soft tissue allograft culture. No significant association was found between a positive culture and surgical site infection ($P = 0.43$).
Conclusion: There was no apparent association between positive intraoperative irradiated soft tissue allograft cultures and surgical site infection in reconstructive knee surgery.

1. Introduction

Higher levels of sports participation in the general population have led to a higher incidence of ligamentous knee injuries and, consequently, an increased use of soft tissue allografts for reconstructive surgery [1–3]. A survey in 2006 regarding anterior

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cruciate ligament (ACL) reconstruction in the United States showed an increase in the proportion of allografts used in a five-year period: from 12% to 22%. A more recent study analyzed a national registry of 16,192 ACL reconstructions performed between 2005 and 2011, and showed that allografts were used in 42.4% of primary cases and 78.8% of revision cases [4,5]. Allografts have a number of advantages over autograft tissue: lack of donor site morbidity, unlimited sizes, shorter operative times, smaller incisions, and a lower incidence of postoperative arthrofibrosis [6]. However, some disadvantages include slower graft incorporation rates, higher rates of graft failure, lower functional outcome scores and patient satisfaction, greater knee laxity, and the risk of disease transmission (including viral or bacterial diseases) [7–11]. Cases of septic arthritis and death due to different *Clostridium* and *Streptococcus* species have been reported in the literature [12–15]. This prompted the routine intraoperative culture of allograft tissues to detect and treat patients with highly virulent and potentially lethal bacteria [16].

The prevalence of positive intraoperative allograft culture results in knee reconstructive surgery has been found to be between 2.6% and 13.3% [6,17–20]. In these studies, low virulent bacteria were mostly identified and patients did not significantly develop higher rates of infection. However, the main weaknesses of these studies were the short follow-up periods and the fact that asymptomatic cases with positive culture results were treated with a full course of antibiotics. Since the Centers for Disease Control and Prevention (CDC) defines an allograft-associated infection as one that occurs within 12 months of implantation [21], longer follow-up periods without the use of prophylactic antibiotic treatment are required to identify infection in patients with low virulent organisms.

The objective of the current study was to determine the prevalence of positive soft tissue allograft cultures in reconstructive knee surgery, and evaluate its association with clinical infection. It was hypothesized that positive intraoperative allograft culture results are not associated with higher postoperative infection rates.

2. Methods

This was a retrospective study that was carried out in a private Level I trauma center with a workers' compensation population. The electronic records of 210 patients who underwent knee reconstructive ligament surgery with the use of at least one allograft between January 2013 and July 2017 were analyzed. Eligibility criteria included any reconstructive knee ligament procedure, including revision surgeries, with an allograft culture sample taken intraoperatively, and a minimum follow-up of 12 months. Patients were excluded if they did not have intraoperative cultures taken and if they did not attend postoperative follow-up visits. After revision of the electronic records, 202 patients were eligible for final analysis, including 170 (84.9%) males and 32 (15.9%) females, with an average age of 38.6 years (SD $\pm$ 11.48).

All grafts were supplied by two tissue banks accredited by the American Association of Tissue Banks (AATB), which have strict policies for serologic and microbiologic testing in accordance with Food and Drug Administration regulations. Blood cultures, as well as tests for human immunodeficiency virus (HIV), syphilis, hepatitis B and C were performed on all donors. All allografts were obtained under sterile conditions at the time of procurement, received low-dose irradiation (<2 mRad), were stored inside two sealed envelopes, and frozen at −80 °C.

In the operating room, the first sealed envelope was opened and then the second envelope inside was thawed at room temperature for 30 min in 500 ml of saline. Afterwards, this envelope was opened and a small amount of tissue from the allograft was obtained for Gram testing and soft tissue cultures (including aerobic and anaerobic organisms). All patients received one gram of cephazolin (or 600 mg of clindamycin in cases of penicillin or cephalosporin allergies) 30 min prior to the incision and then three additional doses to complete 24 h of prophylaxis. This antibiotic protocol is followed at the current institution for cases in which an implant is placed in the patient and is kept overnight in the hospital. Since it does not perform ligamentous reconstructions in an outpatient setting, every patient received the same course of antibiotics.

Intraoperative culture results were obtained from a microbiologist, and the diagnosis of surgical site infection during follow-up was recorded. This was considered if the attending orthopedic surgeon had suspected a surgical site infection, according to subjective clinical parameters and objective laboratory work-up, and if the patient had received antibiotic treatment after diagnosis.

Table 1
Knee surgeries and number of allografts used for ligament reconstruction.

Surgery	Number of allografts
Multiligamentary reconstruction	143 (47.6%)
ACL reconstruction	108 (36.0%)
PMC reconstruction	23 (7.6%)
PCL reconstruction	16 (5.3%)
PLC reconstruction	4 (1.3%)
Extensor mechanism reconstruction	4 (1.3%)
Patellofemoral ligament reconstruction	2 (0.6%)

ACL, anterior cruciate ligament; PCL, posterior cruciate ligament; PLC, posterolateral corner; PMC, posteromedial corner.

The primary outcome of this study was the presence of surgical site infection during the follow-up period and its association with a positive intraoperative allograft culture result. As a secondary outcome, the microorganisms identified in positive cultures and the type of allograft used were listed.

A priori power analysis was performed. It was calculated that to obtain 80% power with a probability of <5% for a Type I error, 55 patients with positive cultures were necessary. Fisher's Exact test was performed to determine association between positive culture results and postoperative infection. Significance value was set at $P < 0.05$. Stata version 12.0 was used for all statistical analysis. Institutional review board approval was obtained prior to the beginning of the study.

3. Results

Three-hundred allografts were implanted in 202 patients and were followed-up for an average of 32.9 ± 12.5 months (range 13 to 57.9) (Table 1).

Sixteen patients (7.9%) had positive intraoperative allograft cultures (Table 2). Five were Achilles tendon allografts with bone blocks and 11 were soft tissue allografts. The most frequently isolated organism was *Bacillus* species (six cases), followed by *Staphylococcus epidermidis* (four cases) and *Staphylococcus hominis* (two cases). Two positive cultures for methicillin-sensitive *Staphylococcus aureus* (MSSA) and one case of methicillin-resistant *Staphylococcus aureus* (MRSA) were identified (Table 3). All of them received standard prophylactic antibiotics and did not receive any additional treatment after the positive culture result. At final follow-up, none of them had developed a surgical site infection nor had required knee fluid aspiration. One patient was revised due to persistent knee instability. On the other hand, in the group of patients with negative intraoperative cultures, nine (4.5%) developed a surgical site infection that required antibiotic therapy (none had cultures of knee fluid aspiration) and one (0.5%) developed septic arthritis that required arthroscopic knee lavage and allograft debridement. Postoperative cultures were positive for *Staphylococcus capitis* in this patient.

Fisher's Exact test did not demonstrate a statistically significant association between the presence of a positive allograft culture and the development of surgical site infection ($P = 0.43$).

4. Discussion

This study showed that there was no significant association between a positive intraoperative soft tissue allograft culture and the development of surgical site infection at an average follow-up of 32.9 months. Previous studies, mainly in isolated ACL reconstruction surgeries, have shown similar results in shorter-term follow-up periods and with confounding factors such as the use of antibiotic treatment in asymptomatic patients with positive culture results [6,17–19,22]. The possibility of an infection occurring after 12 months of surgery, due to the presence of low virulent microorganisms, is currently unclear. This study sought to provide a case series with a minimum follow-up of 12 months and the absence of antibiotic treatment in patients with positive culture results.

Contamination of allograft tissue may occur at any time between procurement and final implantation. Factors potentially associated with this risk include a prolonged time frame between donor death and tissue procurement, a deficient screening protocol of the donor tissue, an inadequate sterilization or storage technique, and incorrect manipulation of the allograft tissue at the time of surgery. This makes it very difficult to determine when the tissue was contaminated. Joyce et al. published an informative scientific exhibit on the use of allograft tissue after numerous reports of *Clostridium*-derived infections. They recommended obtaining intraoperative cultures of allografts before implantation, in order to detect potentially highly virulent organisms [23]. Although a positive culture does not warrant graft removal, it does enable monitoring of the sterility of allografts at the time of implantation. This gives the treating physician some feedback on the sterility process during procurement, preparation and implantation.

A few authors have studied bacterial contamination in allograft tissue and its implications in knee ligament reconstructive surgery. Tomford et al. performed an early report of infection rates after utilizing allograft tissue. They found a five percent infection rate with bony allografts used in the treatment of bone tumors and correlated the risk of infection to the size of the allograft [24]. Deijkers et al. reported the contamination rate of 2000 bone allografts after procurement and rinsing with antibiotics, demonstrating that 50% of allografts were contaminated with low virulent organisms (skin commensals) and three percent with more virulent bacteria (gastrointestinal commensals) [25]. Centeno et al. prospectively cultivated 210 samples of soft tissue allografts used for ACL surgery, and had a positive culture rate of 4.8%. Three patients, 50% of their cultures, were positive for highly virulent microorganisms

Table 2
Comparison between patients' intraoperative culture results and the development of a postoperative infection in each group.

	Positive culture	Negative culture
With postoperative infection	0	10
Without postoperative infection	16	176
Total number of patients	16	186

$P = 0.43$ for independence Fisher's Exact test.

Table 3
Patients with a positive intraoperative culture.

Patient number	Organism identified	Allograft type
1	*Bacillus* species	Peroneus longus
2	*Bacillus* species	Peroneus longus
3	*Bacillus* species	Peroneus longus
4	*Bacillus* species	Achilles
5	*Bacillus* species	Achilles
6	*Staphylococcus epidermidis*	Achilles
7	*Staphylococcus epidermidis*	Peroneus longus
8	*Staphylococcus epidermidis*	Peroneus longus
9	*Staphylococcus epidermidis*	Achilles
10	MSSA	Peroneus longus
11	MSSA	Peroneus longus
12	*Staphylococcus hominis*	Achilles
13	*Staphylococcus hominis*	Tibialis posterior
14	MRSA	Semitendinosus
15	*Staphylococcus warneri*	Peroneus longus
16	*Bacillus* species and *Leifsonia aquatica*	Peroneus longus

Results show the type of bacteria identified and the type of tendon allograft used. None developed a postoperative knee infection. MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus.*

[17]. Diaz de Rada et al. reconstructed 181 ACLs with bone–patellar tendon–bone allografts and showed that 13% of them had positive cultures that did not go on to develop an infection. However, in these cases they gave antibiotic treatment to all patients with positive cultures and recommended treatment with oral antibiotics for two weeks [18]. Guelich et al. presented a case series of 237 ACL reconstructions with an average follow-up of 7.5 months and showed a 9.7% positive culture rate without development of infection [22]. Fowler et al. also presented a case series of ACL reconstructions with positive cultures and without subsequent infections. However, their rate of positive cultures was the lowest reported (three cultures out of 115 patients) [19]. Lastly, Phornphutkul et al. studied allograft contamination in 105 cases, with six positive cultures of bone–patellar tendon–bone allograft samples (5.7%). They suggested that the bony component could make sterilization of the graft more difficult [6].

The current study showed a positive intraoperative culture prevalence of 7.9%, in which none of the patients developed a surgical site infection. This could be explained by profuse irrigation during surgery, use of antibiotics in the perioperative period, and presence of low virulent microorganisms. As was mentioned previously, the rationale for completing 24 h of antibiotic prophylaxis was due to a standardized protocol at the current institution. It is believed that this has not proven to decrease rates of surgical site infection compared to a single pre-operative dose. The protocol has now been modified, and no more than a single pre-operative dose is given in cases with isolated ligament reconstructions. As for the presence of low virulent microorganisms, it was noticed that six of 16 cultures were positive for *Bacillus* species, an aerobic spore-forming rod that stains Gram positive or Gram variable. Except for a few species, the large majority have no pathogenic potential and are not associated with disease in humans or animals. They are widely distributed in the environment, although their main habitat is the soil. This may suggest that contamination occurred during allograft procurement, processing, storage, sample taking or analysis in the laboratory. In the authors' opinions, it is possible that one of the main sources of contamination was during the procedure itself. The current study identified a diverse population of microorganisms that differed from those identified in previous studies, which could have reflected the hospital's microorganism epidemiology.

Other strategies looking to decrease rates of infection in ligamentous surgery have been proposed. Recent studies have demonstrated statistically significant reductions of knee infections after autograft pre-soaking with vancomycin in combination with intravenous antibiotics [26–28]; however, none of them used allografts. Future studies with vancomycin pre-soaked allografts are necessary to establish if this might reduce positive culture rates and clinical knee infections in this group.

The main advantages of the current study were its large sample size, the avoidance of antibiotic treatment in patients with positive culture results, and the longer follow-up period. The latter was possible because the current hospital works with a captive patient population. If a worker suffers a knee trauma during working hours, they will receive free medical attention and treatment at the hospital for their entire life, as long as they attend their scheduled follow-ups.

On the other hand, one of the main weaknesses was that after performing a power analysis, it was noted that to accept $<5\%$ probability of a Type I error and a power of 80%, the sufficient sample size would have to include at least 55 patients with positive intraoperative cultures. Since the current hospital began to implement routine intraoperative allograft culture in 2013, it has managed to identify 16 positive cultures in a four-year period, which means that it would require a minimum of 12 years of analysis to obtain the necessary number of patients. Another limitation of the results was that all patients received three postoperative doses of antibiotics. This might not be representative of the common postoperative management of isolated ligament reconstruction surgeries, as many centers perform these cases as outpatient procedures and patients receive a single dose of antibiotics. This factor could have decreased the incidence of postoperative graft infection, but cannot be proven with current data. It is believed that antibiotic prophylaxis in the perioperative period should have less of an impact on the incidence of postoperative infection compared to empirically treating patients with positive culture results. Another weakness of this study was that it did not perform joint fluid aspiration in knees with positive cultures, which could have helped to identify false positive culture results. This was not done because all the patients in this group remained clinically asymptomatic. In addition, it did not include non-irradiated grafts, and therefore the results should only be considered specifically for patients with irradiated grafts, even though a recent study has shown no differences in rates of

infection between the use of processed and non-processed allografts [29]. Finally, contrary to what other reports have shown, it did not detect highly virulent bacteria [17,22]. This could suggest that patients with low virulent microorganisms grown in intraoperative cultures do not develop surgical site infection even after >12 months' of follow-up.

5. Conclusion

There was no apparent association between positive intraoperative irradiated soft tissue allograft cultures and surgical site infection in reconstructive knee ligament surgery.

Declaration of interest statement

Gonzalo Ferrer and Gonzalo Espinoza are paid consultants for Smith & Nephew.
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