Investigation of Postoperative Allograft-Associated Infections in Patients Who Underwent Musculoskeletal Allograft Implantation

Christine Crawford,1,2,a Marion Kainer,2,a Daniel Jernigan,2 Shailen Banerjee,2 Carol Friedman,3 Faruque Ahmed,3 and Lennox K. Archibald2,a
1Epidemic Intelligence Service, Division of Applied Public Health Training, Epidemiology Program Office, 2Division of Healthcare Quality Promotion, and 3Epidemiology Program Office, Division of Prevention Research and Analytic Methods, Centers for Disease Control and Prevention, Atlanta, Georgia

Background. The rate at which allografts are used in surgical procedures has doubled in the United States during the past decade. In 2002, one outpatient surgical center (SC-X) identified a cluster of surgical site infections (SSIs) after anterior cruciate ligament reconstructive surgery (ACLRS). Therefore, we conducted an investigation to determine the extent of the outbreak and to identify risk factors.

Methods. Our investigation included retrospective cohort and observational studies. A case patient was defined as any patient who acquired a SSI after undergoing ACLRS at SC-X between February 2000 and June 2002 (the study period). Data collected included demographic characteristics, clinical information, and graft details, such as processing method (i.e., aseptic or sterile).

Results. Of 331 patients who underwent ACLRS during the study period, 11 (3.3%) met the case definition. All infections occurred at the tibial fixation site of the graft and involved 8 different microorganisms; the median time to a positive culture result was 55 days after ACLRS. The infection rate for patients who received aseptically processed allografts was 4.4% (11 of 250 patients), compared with 0% (0 of 81) for patients who received autografts or sterile allografts (P = .07). Use of a supplementary staple for tibial fixation, compared with other fixation methods that did not involve such staples, increased the risk of infection 10-fold in univariate analysis (relative risk [RR], 10.0; 95% confidence interval [CI], 3.0–32.9) and 9-fold when controlling for tissue processing method (RR, 9.0; 95% CI, 2.8–28.8).

Conclusions. The use of sterile allograft tissue appears to be associated with a significant reduction in the risk of postoperative infection, particularly in the presence of adjunctive fixation. Larger clinical studies are necessary to confirm this observation.

According to the American Association of Orthopedic Surgeons, doctors in the United States treat ∼95,000 anterior cruciate ligament (ACL) injuries each year, with an estimated cost of $1 billion [1]. ACL injuries usually occur when persons come to a quick stop with a sudden change in direction of body motion, especially during sporting activities, such as basketball, football, skiing, or soccer, or if the knee is hyperextended. ACL injuries may be more frequent among women than men [2].

ACL reconstruction involves the replacement of the injured ACL with a tendon graft procured from 1 of 2 sources: autografts, which are obtained intraoperatively from the patient undergoing the reconstructive procedure, and allografts, which are recovered from human cadavers. After recovery from a cadaver, allografts undergo either aseptic processing or sterilization. During aseptic processing, precautions taken to minimize the introduction of new organisms during tissue recovery from cadavers involve processing in a controlled environment by means of validated methods to prevent contamination of the allograft tissue with environmental microorganisms. After the recovery stage, tissues might be further treated with chemicals or antimicrobials to eliminate bacteria. However, the tissue is not
sterilized and still may harbor microorganisms that originated from the cadaver. During sterilization, aseptically processed tissue is further treated with a method that greatly reduces contamination with bacteria, mycobacteria, viruses, fungi, and spores to benchmarked levels that do not pose a meaningful risk of infection. Approximately 850,000 allografts were distributed in the United States in 1999, compared with 350,000 in 1990 [3].

BACKGROUND

Surgical center X (SC-X) is an outpatient surgical center in California. SC-X opened in February 2000 and houses 5 operating rooms. Surgical procedures are performed Monday through Friday from 5:30 a.m. until 5:00 p.m. SC-X records indicated that, as of 31 March 2002, more than 10,000 procedures had been performed, 40% of which were orthopedic. The first case of surgical site infection (SSI) at SC-X was reported in December 2000, and by March 2002, a total of 10 SSIs had been ascertained. SC-X personnel investigated these 10 SSIs and initially came to the conclusion that SSI risk might be associated with use of the Intrafix device (Mitek Products) used to fix allograft tissue to the tibia. Therefore, SC-X discontinued use of the Intrafix device in May 2002. However, because intrinsic contamination of the allografts could not be ruled out, the Centers for Disease Control and Prevention (CDC) was invited to assist in an epidemiologic investigation to determine the extent of the outbreak and identify risk factors for SSI in patients who had undergone orthopedic procedures, to ascertain the role of allograft tissue as a risk factor for SSI, and to control and prevent the acquisition of SSIs in patients who undergo orthopedic procedures and allograft implantation.

PATIENTS AND METHODS

Determination of infection rates. We calculated and compared the infection rate for orthopedic procedures versus that for nonorthopedic procedures from SC-X’s opening in February 2000 through June 2002 (the study period). Infection rates after the performance of ACL reconstructions and other orthopedic procedures were then calculated and compared.

Case definition and ascertainment. A case patient was defined as any patient who acquired an SSI after ACL reconstructive surgery performed at SC-X between February 2000 and June 2002. We used the National Nosocomial Infections Surveillance System definitions for incisional and deep surgical wounds [4]. Allograft recipients were considered to have acquired an allograft-associated infection if at least 1 of the following events occurred or were present at the surgical site ≤1 year after the operative procedure: purulent drainage; isolation of an organism from a culture of drainage fluid; wound dehiscence or requirement for wound opening by the surgeon, unless a wound specimen was culture negative; detection of an abscess or other evidence of infection on direct examination, during surgery, or by histopathologic examination; and diagnosis of infection by the surgeon or attending physician [4].

Cases were ascertained through an internal quality assurance surveillance program at SC-X. At the end of each quarter, the SC-X director sends each SC-X-affiliated surgeon a list of the patients seen by the surgeon during that quarter. The surgeons then report any infections observed in their patients. The mean response rate among surgeons for this quality assurance program is 85.8%. During the most recent quarter before the CDC investigation, the response rate was ~99%.

Assessment of ACL reconstructive surgical procedures and personnel interviews. During our investigation, we observed several surgical procedures, methods of fixation of the allograft tendons, and types of devices used. To document infection-control practices and procedures before and during surgical procedures, we interviewed orthopedic surgeons, physician assistants, SC-X nurses, and the infectious diseases specialist who consulted on 4 of the 11 cases.

Epidemiologic study. To identify risk factors for SSI after ACL reconstructive procedures performed since the opening of SC-X, we conducted a retrospective cohort study. We recorded patient demographic characteristics, surgery details, complications (if any), graft and donor details, SSI characteristics, previous arthroscopic procedures or ACL reconstructive surgery, and types of femoral or tibial fixation devices used in previous surgical procedures. Graft details collected included type and size of the graft and whether an allograft or an autograft was involved. In addition, we collected information about the operating surgeon and operating room. For each SSI ascertained, we documented the causative microorganism and the time from specimen collection to positive culture result. A tissue trace-back investigation was conducted to identify the tissue processor, graft serial number, donor number, and allograft processing methods. Cases were then classified according to the processing method (i.e., aseptic processing vs. sterilization). Sterile allografts and autografts were grouped together, because they were expected to have the same level of infection risk.

Statistical analysis. Univariate and multivariate analyses of the data were conducted using Epi Info, version 6.04 (CDC; Atlanta, GA), SAS, version 6.12 (SAS Institute), and Stat Exact, version 5 (Cytel Software). Fisher’s exact test was used to compare categorical variables. Where zeros were present in the cells of 2 × 2 tables, Woolf’s method was used to approximate the measures of association. Relative risks (RRs), ORs, and 95% CIs were calculated.

RESULTS

Infection rates. During the study period, the overall crude SSI rate was 0.25%. The SSI rate after orthopedic surgical pro-
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Table 1. Demographic characteristics and results of microbiologic analyses for 11 patients with surgical site infections after anterior cruciate ligament reconstructive surgery in an outpatient surgical center, California, 2002.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, years</th>
<th>Pathogen(s) recovered from tissue</th>
<th>Time to positive culture result, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>40</td>
<td>Candida glabrata</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>44</td>
<td>Staphylococcus aureus</td>
<td>138</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>35</td>
<td>Staphylococcus species</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>62</td>
<td>No growth</td>
<td>...</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>38</td>
<td>Enterococcus faecalis</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>17</td>
<td>Staphylococcus and Enterococcus species</td>
<td>62</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>31</td>
<td>Serratia liquefasciens</td>
<td>24</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>27</td>
<td>Enterobacter agglomerans</td>
<td>28</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>33</td>
<td>Escherichia coli</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>27</td>
<td>C. glabrata</td>
<td>91</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>46</td>
<td>Enterococcus species</td>
<td>48</td>
</tr>
</tbody>
</table>

Table 2. Demographic and clinical characteristics of 331 patients who underwent anterior cruciate ligament reconstructive surgery in an outpatient surgical center, California, 2002.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient demographics</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>129 (39.0)</td>
</tr>
<tr>
<td>Male</td>
<td>202 (61.0)</td>
</tr>
<tr>
<td><strong>Knee</strong></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>159 (48.0)</td>
</tr>
<tr>
<td>Right</td>
<td>172 (52.0)</td>
</tr>
<tr>
<td><strong>Graft</strong></td>
<td></td>
</tr>
<tr>
<td>Autograft</td>
<td>41 (12.4)</td>
</tr>
<tr>
<td>Allograft</td>
<td>290 (87.6)</td>
</tr>
<tr>
<td>Sterile processing</td>
<td>40/290 (13.8)</td>
</tr>
<tr>
<td>Aseptic processing</td>
<td>250/290 (86.2)</td>
</tr>
<tr>
<td><strong>Tendon used for graft</strong></td>
<td></td>
</tr>
<tr>
<td>Tibialis</td>
<td>266 (80.4)</td>
</tr>
<tr>
<td>Other (e.g., hamstring and patellar tendon)</td>
<td>65 (19.6)</td>
</tr>
<tr>
<td><strong>Tibial fixation</strong></td>
<td></td>
</tr>
<tr>
<td>Intrafix only</td>
<td>207 (62.5)</td>
</tr>
<tr>
<td>Other</td>
<td>124 (37.5)</td>
</tr>
<tr>
<td><strong>Staple use</strong></td>
<td></td>
</tr>
<tr>
<td>With another tibial fixation device</td>
<td>12 (3.6)</td>
</tr>
<tr>
<td>None</td>
<td>319 (96.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Proportion (%) of patients with infection</th>
<th>Relative risk (95% CI)</th>
<th>P (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allograft</td>
<td>11/290 (3.8)</td>
<td>3.3(^b) (0.4–∞)</td>
<td>.37</td>
</tr>
<tr>
<td>Autograft</td>
<td>0/41 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aseptic</td>
<td>11/250 (4.4)</td>
<td>70.5(^b) (1.1–∞)</td>
<td>.07</td>
</tr>
<tr>
<td>Autograft or sterile allograft</td>
<td>0/81 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibial fixation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrafix only</td>
<td>8/207 (3.9)</td>
<td>10.6 (.4–5.9)</td>
<td>.55</td>
</tr>
<tr>
<td>Other</td>
<td>3/124 (2.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staple use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With another tibial fixation device</td>
<td>3/12 (25)</td>
<td>10 (3.0–32.9)</td>
<td>.005</td>
</tr>
<tr>
<td>None</td>
<td>8/319 (2.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) By Fisher’s exact 2-sided test
\(^b\) By Woolf’s method.

in addition to another fixation device, such as the Intrafix tibial screw or an interference screw (table 2).

None of the patient demographic data or variables, such as age, sex, or comorbid conditions, were associated with an increased risk of infection. In addition, operating room, right or left knee, surgeon, previous ACL reconstructive surgery, previous arthroscopy, method of femoral fixation, and tissue processor were not associated with infection. Recipients of aseptically processed allografts were at higher risk of acquiring SSI than were recipients of sterile allografts or autografts (11 of 250 vs. 0 of 81 patients; \(P = .07\)). All case patients were recipients of aseptically processed tissues (table 3). Use of an Intrafix device alone was not associated with an increased risk of infection (RR, 1.6; 95% CI, 0.4–5.9; \(P = .55\)). However, patients whose tibial fixation included use of a staple with the Intrafix device or interference screw were 10 times as likely to develop an infection than were patients who underwent any other method of tibial fixation (table 3).

Next, we conducted stratified analyses to control for staple use. The increased risk of infection associated with use of a staple remained when controlling for type of processing method. For aseptically processed tissue, the risk of infection when an ACL surgical procedure involved use of a staple in conjunction with another tibial fixation device was 9 times the risk associated with use of any other fixation devices (table 4). We then constructed a multivariate logistic regression model that included method of processing and staple use as variables; estimates were made for the adjusted effect of each of these risk factors. An analysis controlling for staple use revealed that use of nonsterilized tissues was 3 times more likely to result in an infection than was use of sterilized tissues, although this finding was not statistically significant (OR, 3.1; 95% CI, 0.6–


<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Proportion (%) of patients with infection</th>
<th>Relative risk (95% CI)</th>
<th>P (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue processing method stratified by staple use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staple with another tibial fixation device</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allograft</td>
<td>3/10 (30)</td>
<td>1.9(^b) (0.2–∞)</td>
<td>1.0</td>
</tr>
<tr>
<td>Autograft or sterile allograft</td>
<td>0/2 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No staple</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allograft</td>
<td>8/240 (3.3)</td>
<td>5.7(^b) (0.7–∞)</td>
<td>.20</td>
</tr>
<tr>
<td>Autograft or sterile allograft</td>
<td>0/79 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staple use stratified by tissue processing method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aseptic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staple with another tibial fixation device</td>
<td>3/10 (30)</td>
<td>9.0 (2.8–28.8)</td>
<td>.006</td>
</tr>
<tr>
<td>No staple</td>
<td>8/240 (3.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autograft or sterile allograft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staple with another tibial fixation device</td>
<td>0/2 (0)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>No staple</td>
<td>0/79 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) By Fisher’s exact 2-sided test
\(^b\) By Woolf’s method.

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conclusively demonstrate this because zeros were present in the
factor in future studies. In our analysis, we were not able to
being said, bacterial contamination of allograft tissue that has
contamination of the allograft alone was plausible but was not
tenth the rate for ACL procedures. Second, intrinsic
claved, and the infection rate for those procedures was one-
tenth the rate for ACL procedures. Second, intrinsic
association between SSI and internal fixation of allograft tissue
at the tibial site.
We considered several hypotheses to account for SSI occur-
rence. First, inadequate autoclave processing of the staples was
possible—staples were the only tibial fixation devices that were
not supplied sterilized by the manufacturer. We feel that this
was unlikely, because other screws not used in ACL reconstruc-
tion but used in other orthopedic procedures were also auto-
claved, and the infection rate for those procedures was one-
tenth the rate for ACL procedures. Second, intrinsic
contamination of the allograft alone was plausible but was not
completely supported by the results of our investigation. That
being said, bacterial contamination of allograft tissue that has
not been sterilized is possible and should be considered a risk
factor in future studies. In our analysis, we were not able to
conclusively demonstrate this because zeros were present in the
cells of 2 × 2 tables and because of the mathematical adjust-
ments made to mitigate these drawbacks. Third, insertion of
fixation hardware of any type or material is usually performed
at the conclusion of the arthroscopically assisted allograft re-
constructive procedure. Passage of an internal fixation device,
such as a staple or interference screw, through a surgical site
that is contaminated with bacteria is likely to be associated with
a higher risk of postoperative infection. If the hypothesis is true
that placement of an internal fixation device adjacent to allo-
graft tissue that has not undergone a secondary sterilization
process is likely to predispose to infection, then one would
expect infections to also occur after procedures involving al-
graft fixation at the femoral implantation site. In our inves-
tigation, no SSIs were associated with femoral fixation. This
could be due to the fact that the tibial insertion site has sub-
stantially less soft and vascular tissue, compared with the fem-
oral site, rendering the former more prone to infection. This
hypothesis needs to be explored further, because >25% of sur-
geons generally prefer a combination of one of the fixation
methods described above and adjunct stapling for tibial fixation
of the graft, depending on the type of tendon used to replace

DISCUSSION
Our investigation highlighted several issues relevant to tissue
transplantation safety: (1) SSIs did not occur among recipients
of autografts or allograft tissue that had undergone a steriliza-
tion process; (2) the infection rate among recipients of al-
lografts that were not sterilized was 4.4%; (3) the use of sterile
allograft tissue appears to be associated with a significant re-
duction in the risk of postoperative infection, particularly in
the presence of adjunctive fixation; and (4) there is a potential
association between SSI and internal fixation of allograft tissue

The heterogeneity of the organisms isolated from case pa-
patients suggests that infections did not arise from a point source,
a common source, or a specific donor. This finding also sup-
ports the hypothesis that bacterial contamination either oc-
curred at the surgical site for passage of the arthroscope or
ensued after inadequate elimination of microorganisms during
tissue processing and use of a supplementary staple at the tibial
fixation site. Because our epidemiologic data suggested a sig-
nificant role for staples, we believe that the outbreak is most
plausibly explained by a mechanism that involved the adjunct
use of staples in procedures in which either allograft tissue that
did not undergo secondary sterilization was used or the staple
was passed arthroscopically through a surgical site that was in-
advertently contaminated with bacteria. Also, the use of a staple
could have resulted in inflammation and necrosis at the fixation
site on the tibia. Repeated trauma, such as that due to kneeling
or to pulling the tendon too tightly by the supplementary staple,
might have caused inflammation and necrosis, thereby providing
a milieu for bacterial proliferation. This may explain the relatively
long time to positive culture results for case patients (on average,
3 months after the procedure) and the predominance of infec-
tions after tibial versus femoral fixation.

Our study had several limitations. First, the lack of variability
of surgical practices at SC-X reduced the statistical power of the
analyses. Second, the practices at SC-X were not generalizable to
all practices in the United States. For example, almost 90% of
the surgical procedures at SC-X used allografts, whereas most
surgical practices in the United States currently use autografts
[5]. Third, there might have been a reporting bias: SC-X’s sur-
veillance system for SSI relied entirely on self-report by surgeons,
and it is likely that some surgeons were reluctant to report rou-
tinely about adverse events associated with surgical procedures
for which they were responsible. Although surgeons who know-
ingly used aseptically processed grafts might be biased in re-
porting infections in their patients, we believe this was unlikely
at SC-X, because none of the surgeons we interviewed were aware
of the processing methods used for the tissues they used in ACL
procedures. Fourth, underreporting of staple use could have oc-
curred, because the staple is the only fixation device without a
label that can be affixed to the surgical record.

The 4.4% rate of infection among recipients of aseptically
processed allograft tissue is consistent with the results from a
previously published study that documented a 5% rate of in-
fec tion associated with musculoskeletal allografts that were not
sterilized [6]. Because the majority of surgeons perform >90% of
their ACL reconstructions as outpatient procedures, allo-
graft-associated infections will not be detected by traditional
nosocomial surveillance methods [5]. Our investigation was greatly facilitated by SC-X already having an SSI surveillance system in place and by the availability of information regarding the processing method for each allograft.

The need for sterilization of musculoskeletal allografts has recently been highlighted [7–9]. γ Irradiation or ethylene oxide was historically used to sterilize tissues. However, γ irradiation at high doses tends to adversely affect the biomechanical properties of collagen, rendering the tissue mechanically unsound and, therefore, inappropriate for use. Although ethylene oxide sterilization does not affect the biomechanical properties of the tendon, it is associated with postoperative synovitis [10, 11]. Because of these inherent problems with γ irradiation and ethylene oxide, new tissue sterilization methods, such as low-temperature chemical sterilization, have been developed [8, 12].

Many patients benefit from musculoskeletal allograft implants. However, infections from tissues processed with methods that do not significantly decrease the microbial burden may occur and result in poor outcomes, including death [7]. Case surveillance by the CDC identified at least 26 additional cases of allograft-associated infections, of which 70% occurred in patients who had undergone ACL reconstructive surgery. Eighty-one percent of the allografts were not sterilized [7]. Findings from these investigations have led to changes in federal regulations and national standards to reduce bacterial contamination of processed allograft tissue [13, 14].

The results of our investigation highlight the need for tissue transplant safety by means of implementation of technologies that sterilize musculoskeletal allograft tissue and for better surveillance activities for allograft-associated infections; patient safety is not assured when allograft tissues have not undergone a sterilization process. Finally, we have highlighted potential risks for allograft-associated infections after arthroscopy-assisted fixation of aseptically processed tissue at the tibial fixation site. Use of adjunct hardware, the site of arthroscope insertion, and the anatomic site of fixation (e.g., tibial vs. femoral) should be considered potential risk factors when investigating allograft-associated infections.

Acknowledgments

We are grateful to the clinicians, patients, and staff at surgical center X and to staff at the California Department of Health who assisted in this investigation. In particular, we are indebted to Dr. Robert Armstrong, who facilitated the conduct of this investigation. Also, we thank the administrators at surgical center X for implementing the recommendations that followed the investigation.

This investigation was conducted and completed while the authors were full-time employees of the Centers for Disease Control and Prevention. The United States Public Health Service underwrote the costs of the entire investigation.

Potential conflicts of interest. L.K.A. is the medical director of Regeneration Technologies. All other authors: no conflicts.

References