Allograft Donor Characteristics Significantly Influence Graft Rupture After Anterior Cruciate Ligament Reconstruction in a Young Active Population

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Background: Graft selection in anterior cruciate ligament (ACL) surgery can be difficult in a young active population given their high rates of reinjury. Allografts allow for control over graft size and reduce morbidity of autograft harvest. There are mixed results about the use of allograft in the literature; however, the influence of the properties of the allograft on outcomes has not been considered.

Hypothesis: ACL reconstruction with allografts from older donors will have a higher rate of graft rupture when compared with allograft from young donors.

Study Design: Cohort study; Level of evidence, 3.

Methods: Patients (N = 211) aged 13 to 25 years underwent primary ACL reconstruction with fresh-frozen nonirradiated allograft. Four graft types were used: patellar tendon, Achilles tendon, tibialis anterior, and tibialis posterior. Details were collected on allograft donor age and sex. At a minimum of 24 months, patients were evaluated for any further injuries and subjective analysis by International Knee Documentation Committee (IKDC) questionnaire.

Results: ACL graft rupture occurred in 23.5%. When grafts were separated into single strand (patellar and Achilles tendon) and multistrand (tibialis anterior and posterior), there was a significantly higher rate of reinjury in the single-strand grafts (29.9% vs 11%; $P = .014$). Grafts from female donors aged $\geq$50 years had significantly higher rates of ACL graft rupture (52.6%; $P = .003$) with increased odds by 6.7 times when compared with grafts from male donors aged $<$50 years. There was no significant difference in mean IKDC scores among the groups based on the age and sex of the allograft donor.

Conclusion: The age and sex of the allograft donor and the morphology of the graft significantly influenced the rate of ACL graft rupture in young active patients. Tendons from female donors aged $\geq$50 years should be avoided given the higher rerupture rates as compared with male donors of any age and younger females.

Keywords: allograft; donor sex; donor age; anterior cruciate ligament reconstruction

Young active patients with anterior cruciate ligament (ACL) rupture are difficult to manage. They have high functional demands that require a strong and stable knee, and they have a significantly high rate of reinjury.[21,25,28] Graft selection in the adolescent age group can be particularly difficult given these patients have not yet reached full maturity.[18] At our institute, as well as throughout Australia, hamstring tendon autografts have been the preferred choice. They, however, are not without morbidity and may not be ideal in a young population.

Hamstring muscles are known to act as a secondary stabilizer of the knee. They offer a protective effect on the ACL to prevent anterior translation of the tibia, and they play a role in knee proprioception. After ACL reconstructive surgery, persistent hamstring muscle atrophy and long-term deficits in strength and range of movement have been demonstrated.[5,16,27] Snow et al[27] found a reduction in hamstring volume of 50% a decade after operation, and they reported a significant reduction in volume in the quadriceps muscles, which correlated with decreased power. This suggests possible long-term muscle dysfunction and changes in knee biomechanics. The second issue with hamstring grafts in a young population is that the size may be insufficient to match their future development and functional demands.[8] Thus, allografts may be a good alternative: they avoid donor site morbidity and allow for better control over graft size.

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TABLE 1
Inclusion and Exclusion Criteria

Inclusion	Exclusion
Age ≤25 y	Age >25 y
Isolated anterior cruciate ligament injury	Other significant ligamentous injury the to index knee
Primary reconstruction	Previous anterior cruciate ligament injury to either knee
No injury to the contralateral knee	Patients seeking compensation for their injury
Consent to use of nonirradiated fresh-frozen allograft	

Furthermore, they result in a smaller wound, faster early recovery, and reduced operative time.

There has been a reluctance to adopt allografts given their poor results in the literature.[6,9,11,19,24] Traditionally, allografts were sterilized with gamma radiation. It is well-recognized that ionizing radiation induces structural damage to the tissue and can limit cell potential to undergo a regenerative process.[26] The physical and biological influence of radiation could influence the strength of the graft when used in ACL reconstruction, resulting in higher failure rates. In a more recent systematic review, Mascarenhas et al[20] found no significant difference in ACL graft reinjury rate, postoperative laxity, or patient-reported outcomes in ACL reconstruction between autograft and nonirradiated allograft.

To the best of our knowledge, the influence of donor characteristics and tendon type used as the graft on the outcome of ACL reconstruction with allograft material has never been evaluated. The purpose of this study was to evaluate outcomes at a minimum of 24 months with fresh-frozen allograft in ACL reconstruction in young patients and to assess the influence of allograft tendon type, donor characteristics, and mode of preparation. The primary outcome variable was ACL graft rupture rate, and the secondary outcome was subjective patient-reported outcomes. We predicted that ACL graft rupture rates with allografts may be influenced by the characteristics of the donor, with higher rates of ACL graft rupture in tendons from older donors. We hypothesized that there would be no difference in ACL graft rupture rates based on the type of tendon used or the method of preparation.

METHODS

Between January 2014 and June 2017, 211 patients between the ages of 13 and 25 years underwent primary ACL reconstruction with fresh-frozen allograft. All reported participating in their respective sports at a competitive level. Inclusion and exclusion criteria are listed in Table 1. Ethical approval was sought and granted by a local human ethics committee (St Vincent's Hospital).

Allografts were obtained from Tissue Bank Victoria or Tissue Bank Queensland (government-regulated bodies). The tissue banks provided details on the donor, including age and sex. None of the grafts were prepared with irradiation. The sterilization process included either a 0.5% chlorhexidine and 70% alcohol wash performed 3 times or 320-μg/mL gentamicin wash stored for 24 hours at 4°C. Both banks used sampling techniques and control swabs to ensure sterility. Additionally, donors were screened before organ donation for potential infectious diseases. Grafts were stored at –40°C until time of use.

Both tissue banks supplied all types of grafts. Four graft types were available: tibialis anterior and tibialis posterior with soft tissue alone and patellar tendon and Achilles tendon with bone blocks. The type of graft used in each patient was not randomly allocated but dependent on availability from the tissue bank as well as what the surgeon thought was most appropriate for the patient. The consensus was to avoid patellar tendon allografts in smaller female patients, as the bone blocks result in drilling larger tunnels. Similarly, patellar tendon grafts were avoided in skeletally immature patients to avoid possible disruption of the physis. Thus, Achilles tendon and tibialis posterior and tibialis anterior tendon were preferentially selected for female patients or smaller male patients, and patellar tendons tended to be reserved for larger male patients.

At the time of surgery, the graft was removed from the freezer and defrosted in warm 0.9% sterile saline. The tibialis grafts were measured and cut to approximately 22 cm and folded in half over a No. 5 suture to form a 2-stranded (multistrand) graft. This suture was used as the leading strand to pull the graft through the tunnel. The

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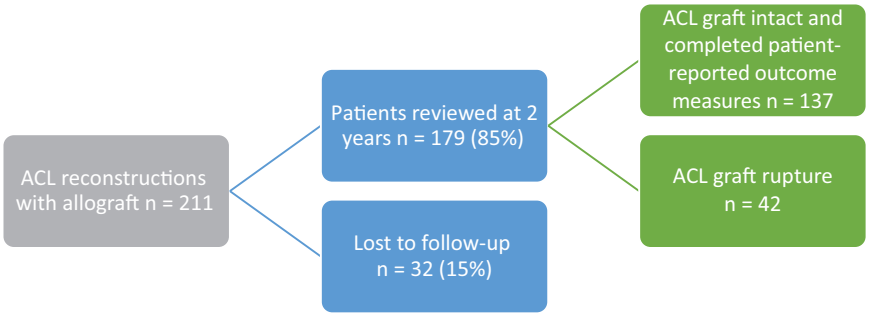

Figure 1. Participant flow. ACL, anterior cruciate ligament.

2 ends of the graft where then sutured with a No. 1 Vicryl whipstitch (Ethicon) for approximately 20 mm. The patellar tendon grafts were made from the central third of the tendon with bone blocks on either end. At either end of the tendon, a 20- to 25-mm trapezoidal bone block was excised, and a 10 mm–wide strip of tendon was cut. The bone blocks were then fashioned to pass through a round graft sizer. Achilles grafts were removed from the bone block and tubularized at both ends with Vicryl whipstitch in an identical fashion to the tibialis tendons. They remained single stranded at the intra-articular component, however. The grafts were all placed in vancomycin-soaked gauze until ready for use.

All operations were performed by 2 orthopaedic surgeons (L.A.P., J.P.R.) using an identical technique. The knee was prepared, and the femoral tunnel was marked with an awl at 5 mm anterior to the posterior capsule insertion at the 11- or 1-o'clock position for the right or left knee, respectively. The femoral tunnel was drilled with the knee in full flexion to the size of the tendon or the bone block previously measured. The tibial tunnel was prepared with a drill guide placed at the footprint of the ACL, one-third of the way along a line from the anterior horn of the lateral meniscus and the medial tibial spine. Fixation at the femur and tibia was with a PEEK RCI HA screw (Smith & Nephew) with the screw 5 to 10 mm from the aperture. All patients underwent a standard accelerated rehabilitation protocol used for all ACL reconstructions at our institute, with postoperative day 1 weightbearing and range of movement exercises. Readiness to return to sports was determined after assessment by the surgeon and physical therapist, typically at 12 months after surgery.

At a minimum of 24 months (range, 24-36 months), patients were assessed with a subjective questionnaire, which included any further knee injuries, return to sports, and the subjective International Knee Documentation Committee (IKDC) knee score.

Statistical analysis was performed with SPSS software for Windows (Version 25; IBM). Statistical significance was set at $P = .05$. Comparison of variables among groups was analyzed with χ^2 tests for categorical data, and comparison of continuous variables was determined by Student t test.

RESULTS

Between January 2014 and June 2017, 211 patients aged ≤25 years underwent primary ACL reconstruction with fresh-frozen allograft, and 179 (85%) were followed up at a postoperative minimum of 24 months (range, 24-36 months). The remaining 32 were unable to be contacted and deemed lost to follow-up. Patient-reported outcomes were measured for 137 patients with intact ACL grafts (Figure 1).

Of the 179 patients, the mean age was 18.6 years (range, 13-25 years), and the majority were male (n = 145; 81%). The most common sports resulting in injury were soccer (n = 75; 35.5%), rugby (n = 49; 23.2%), touch football (n = 26; 12%), netball (n = 15; 7%), and basketball (n = 13; 6%).

For all graft types, 42 patients (23.5%) sustained an ACL graft rupture at a mean of 19 months (range, 5-38 months) after surgery. Of the 42 ACL graft ruptures, only 1 resulted from atraumatic failure. This person received an Achilles tendon allograft from a female donor aged ≥50 years that ruptured at 9 months after surgery. The remaining 41 patients had a graft rupture related to sports or activity. There were 15 graft ruptures within the first year after surgery: 10 related to early return to sports, 1 from jumping at rehabilitation, 1 from change-of-direction drills, 1 fall from skateboarding, 1 fall while skipping, and 1 atraumatic. The Kaplan-Meier curve for ACL graft survival according to donor characteristics is shown in Figure 2, and that according to graft type is shown in Figure 3.

The distribution of the allograft type and rates of ACL graft rupture are shown in Table 2. The most commonly used graft was Achilles tendon (n = 80; 44.7%), followed by patellar tendon and tibialis anterior. Tibialis posterior was the least commonly used. There was a significant difference in the rates of reinjury based on graft type. The patellar tendon had the highest rate of ACL graft rupture (35.1%). When grafts were grouped into single stranded (Achilles and patellar tendons) and multistranded (tibialis anterior and tibialis posterior tendons), there was a significantly higher rate of rupture among the single-stranded grafts (29.9% vs 11%; $P = .014$).

Donor characteristics were divided into 4 groups: male donors <50 years, female donors <50 years, men ≥50 years, and women ≥50 years. Most grafts were from male

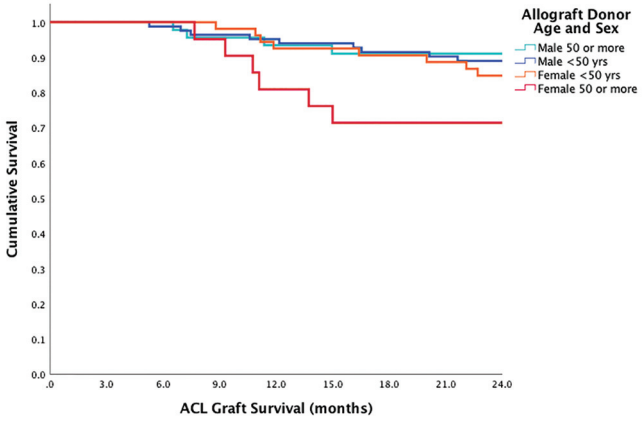

Figure 2. Anterior cruciate ligament (ACL) graft survival according to allograft donor characteristics.

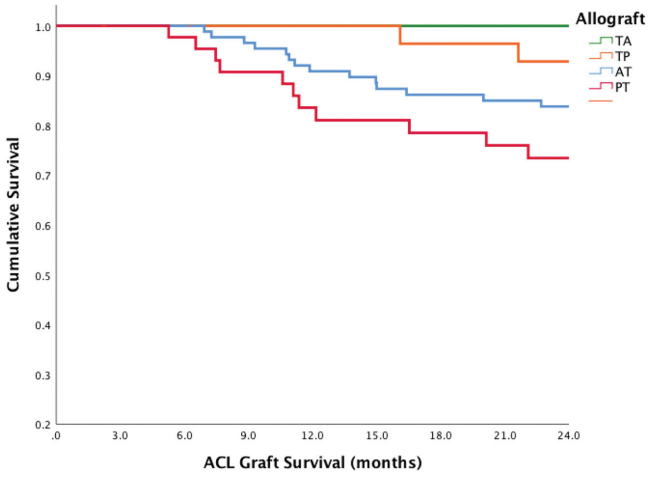

Figure 3. Anterior cruciate ligament (ACL) graft survival according to allograft type. AT, Achilles tendon; PT, patellar tendon; TA, tibialis anterior; TP, tibialis posterior.

donors <50 years old (n = 74), followed by female donors <50 years (n = 45), men ≥50 years (n = 41), and women ≥50 years (n = 19). The rate of rupture was significantly higher when the graft was from women aged ≥50 years (52.6% ACL graft rupture; P = .003) as compared with the other groups. There was no significant difference in graft rupture rates among the other groups (Figure 4). The distribution of grafts from women aged ≥50 years was roughly distributed throughout the graft types: 37% (7/19) Achilles grafts, 16% (3/19) patellar tendon grafts, 21% (4/19) tibialis anterior grafts, and 26% (5/19) tibialis posterior grafts (P = .429).

A multiple regression analysis was performed to assess the relative contribution of the variables of graft type, donor age and sex, and patient age and sex on the rate of ACL graft rupture (Table 3). On multivariant regression analysis, single-strand grafts showed at 3.6-times higher odds of ACL graft rupture (95% CI, 1.2-7.4; P = .014) as compared with multistranded grafts. There was a 6.7-times greater odds of ACL graft rupture when a graft was used from a female donor ≥50 years old (95% CI, 1.9-23.3; P = .003) as compared with those donors <50 years old. Male patients had a 4.6-times greater odds of ACL graft rupture than their female counterparts.

There was no significant difference in rates of ACL graft rupture by mode of preparation (0.5% chlorhexidine + 70% alcohol or 320-μg/mL gentamicin; P = .596).

Graft size was measured in a routine fashion with a sizing tube, as the smallest diameter (in 0.5-mm increments) through which would pass the entire graft, including the bone blocks on the patellar tendon grafts or whipstitched ends of the single-stranded grafts. Given the different shape of the single- and multistrand grafts, a comment on intra-articular graft size cannot be accurately made; the sizing reflects the diameter of the femoral tunnel. The intra-articular component of the tibialis anterior and posterior grafts were measured with mean sizes of 8.77 mm (SD, 1.06) and 8.83 mm (SD, 1.06), respectively. The patellar tendon and Achilles tendon grafts were measured per the bone blocks or whipstitched ends with mean sizes of 10.79 mm (SD, 0.57) and 9.45 mm (SD, 0.89), respectively.

Subjective analysis by IKDC questionnaire was completed by 137 patients with intact ACL grafts. There was no difference in the mean IKDC score across the graft types (analysis of variance, P = .404). There was also no significant difference between graft type and return to very strenuous activities or return to sports at the same level (Table 4). Similarly, there was no significant difference in mean IKDC score or return to very strenuous activities and sports level in the donor characteristics when grouped by age and sex. There was a trend for the group that received grafts from female donors ≥50 years old to have lower rates of return to very strenuous activity (44.4%), but this did not reach significance.

The results indicate that tendon type and donor characteristics are important variables for graft rupture rates. Thus, the preferred allograft may be defined as a multistrand graft from a male donor of any age or a female donor <50 years old. Of the 179 patients, 53 (29.6%) received the preferred graft. ACL graft rupture occurred in 4 of these 53 patients (7.5%), as compared with 38 of 126 (30.2%) who did not receive the preferred graft.

DISCUSSION

This study evaluated the 2-year graft rupture rate for ACL reconstruction with fresh-frozen allografts in a young active population. Although nonirradiated allografts have been shown to be a safe alternative,[30] there remains concern and controversy attributed to the high failure rates reported in the literature.[14,17] However, none of these studies specifically examined the allograft characteristics and their influence on ACL graft rupture rates. This study suggests that the characteristics of the allograft play a significant role in rates of ACL graft rupture—in particular, the age and sex of the donor and the allograft morphology.

The most striking finding of this study was the significantly elevated rate of ACL graft rupture in the allografts

TABLE 2
Graft Type and Rupture Rates[a]

	Achilles	Patellar Tendon	Tibialis Anterior	Tibialis Posterior	Total	*P* Value
Patients	80	37	37	25	179	
ACL graft rupture	22 (27.5)	13 (35.1)	3 (8.1)	4 (16.0)	42 (24)	.027
CACL rupture	7 (8.8)	3 (8.1)	4 (10.8)	3 (12.0)	17 (9.5)	.942

[a]Values are presented as No. (%). ACL, anterior cruciate ligament; CACL, contralateral anterior cruciate ligament.

TABLE 3
Multivariate Analysis of Anterior Cruciate Ligament Graft Rupture by Donor Sex and Age and Patient Age and Graft Type

	No.	Graft Rupture, %	Odds Ratio	95% CI	*P* Value
Donor graft type					
Single-strand graft	117	29.9	3.6	1.3-9.9	.014
Multistrand graft	62	11			
Donor sex and age, y					
Female ≥50	19	52.6	6.7	1.9-23.3	.003
Male ≥50	41	22.0	1.0	0.4-2.9	.942
Female <50	45	20.0	1.1	0.4-3.0	.835
Male <50	74	18.9	Reference		
Patient sex					
Male	145	27	4.6	1.5-14.1	.007
Female	77	6			
Patient age, y					
≤18	114	25	2.2	1.0-4.9	.066
>18	65	22			

from female donors aged ≥50 years, with greater than half of the grafts failing within 2 years. There was a 6.7-times increased odds of rupture if the donor tendon was from a female donor ≥50 years than a donor male <50 years. However, female donors <50 years and male donors of any age performed equally. The question arises of what happens to female collagen as estrogen levels fall and if this makes it an unacceptable graft. Water content throughout the body decreases with aging, as do the concentration of collagen and the rate of collagen synthesis.[2] Blevins et al[3] found a decrease in modulus of elasticity of patellar tendon grafts as donor age increased from 17 to 54 years. Similarly, for the native ACL, cadaveric specimens <35 years old can withstand a load 328% that of cadaveric specimens aged >60 years.[29] The loss of sex steroid hormones in women aged ≥50 years induces sex-related changes in elastin and collagen metabolism.[22] The ACL graft is a collagen matrix with a structural scaffold on which new cells can integrate, vascularize, and ultimately ligamentize.[7] We hypothesize that the hormonal changes in older women may adversely affect the collagen scaffold graft, resulting in an impaired process of ligamentization and subsequently higher rates of ACL graft rupture.

Single-stranded grafts (patellar tendon and Achilles tendon) had higher rates of ACL graft rupture as compared with multistrand grafts (tibialis anterior and tibialis posterior). Grafts were not randomly allocated but dictated by what was available from the tissue bank. There was a period of several months in which tibialis tendons were difficult to obtain from the bank. Additionally, there was an element of selection bias from the surgeon, in that tendons requiring larger tunnel diameters (patellar tendon and Achilles tendon) were used for larger patients; as such, there were significantly more male patients who received single-stranded grafts (102 vs 32 female patients, *P* = .001). Male patients had a higher rate of graft rupture (27% vs 6% female); however, the influence of graft morphology remained significant even when patient sex was controlled for in the regression analysis.

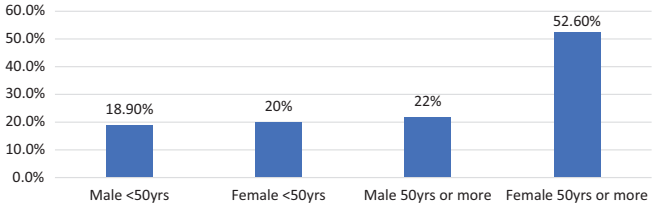

Figure 4. Anterior cruciate ligament (ACL) graft rupture rates by donor characteristic.

When the sex variable is controlled for, the single-strand grafts are still 3.6 times more likely than the multistrand grafts to rupture. Possible explanations for this may include the variations in graft shape: single-strand grafts are ribbon shaped, and multistrand grafts are tubular.

TABLE 4
IKDC Subjective Evaluation and Return to Sports of Patients With Intact Anterior Cruciate Ligament Grafts by Donor Characteristics[a]

	Donor Sex and Age				
	Female ≥50 y	Male ≥50 y	Female <50 y	Male <50 y	*P* Value
Patients	9	32	36	60	
IKDC score[b]	82.1	89.5	89.9	91.6	.120
Participation in very strenuous activities	4 (44.4)	24 (75)	27 (75)	44 (72)	.111
Return to sport at same level	5 (55.6)	17 (53.1)	24 (66.7)	43 (71.7)	.314

[a]Values are presented as mean or No. (%). IKDC, International Knee Documentation Committee.
[b]Out of 100.

This results in a difference in cross-sectional area. A multistrand tubular graft measured as 8 mm will have a cross-sectional area of roughly 50 mm^2; however, a ribbon graft (mean thickness of patellar tendon, 3.54 mm; Achilles tendon, 4.61 mm)[10] will have an area of 28.3 to 36.9 mm^2. It has been recognized that there is a strong positive relationship between maximal load to failure and the cross-sectional area of the graft.[13] Another theory could include the shape of the scaffolding on which new cells can grow. Incorporation of new cells is believed to arise from the periphery of the graft and proliferate down the length of the graft.[15] In a multistrand graft, there are several strands on which cells can travel, as opposed to 1 large strand. This may alter the time to ligamentization and may reflect why, within the first 16 months of our study, all graft ruptures were in single-strand grafts. The lower ACL graft rupture rate in the multistrand grafts could also be a result of which tendon was harvested. The Achilles tendon is recognized to have relatively poor vascularity, with blood supply predominantly arising from the paratenon and musculotendinous junction[1]; comparatively, the tibialis anterior tendon has complete blood supply without any evidence of avascular zones,[23] perhaps affecting the overall strength of the tendon. In vivo, the Achilles tendon and patellar tendon are under significant load and more prone to rupture than tibialis anterior tendons; thus, there may be greater degenerative changes in these high-load tendons. Furthermore, lower ratios of collagen fibrils to interstitium have been shown in the patellar tendon versus other tendon types.[12] Thus, the blood supply in vivo, the load through the tendon, and the architecture of the tendon being used as a graft may be important to consider. This, however, is all speculation, and further research on the properties and structure of the allograft should be considered in the future.

The overall ACL graft rupture rate of the allograft at 2 years was 23.5%. This rate of reinjuries in a population aged <25 years is relatively consistent throughout the literature, regardless of graft type. Wiggins et al[28] found, in a systematic review, that the overall rate of graft rupture was 15%, but for athletes aged <25, the ACL injury rate was 23% within 5 years. Although we did not directly compare with hamstring autograft in this study, previous studies from the same institution may function as a historical match of this patient population, using the same surgeons, surgical technique, and rehabilitation protocol.[4,21] In a cohort of 673 patients with a mean age of 29 years, hamstring tendon autografts were found to have an 11% overall rate of ACL graft rupture over 15 years,[4] and in a study of 288 adolescents, the graft rupture rate was 17% in 15 years.[21] The rate of graft rupture within 2 years was 7% in the adult cohort and 8% in the adolescents. The overall allograft rupture rate in the current study is higher than our autograft cohort; however, when the preferred graft characteristics were targeted (multistranded; male donor or female donor <50 years), the rupture rate of 7.5% at 2 years is consistent with our previous findings with hamstring autografts.

There was no significant difference in IKDC scores by graft type or donor characteristics. However, in the 9 patients with intact grafts from female donors ≥50 years, only 44.4% returned to participating in very strenuous activities, as opposed to 72% to 75% of participants with donor grafts from women aged <50 years or male donors of any age. This appears to be a possible trend, and the low number of patients in the group may prevent it from reaching significance. A larger cohort of patients may help illustrate this difference. A limitation of this study is the lack of objective follow-up to determine clinical laxity and possible attenuation of the graft. Objective evaluation was collected on 89 of the 179 patients, given less than 50% follow up, the results were not formally included in the analysis. Among those examined, there was no significant difference in laxity on KT-1000 arthrometer testing between the donor sex and age or the morphology of the graft ($P = .823$ for age and sex, $P = .415$ for morphology).

With the surgical bias, the short-term follow-up does not indicate how the allograft will perform in the long term. Although most reinjuries do occur within the first 2 years,[25] follow-up would certainly be beneficial to determine the outcomes of allografts in the long term and is planned in this cohort. Furthermore, a more comprehensive evaluation of graft size may reveal a reason for the rates of rupture for the different morphology of the grafts.

Graft choice is a fundamental component of ACL reconstructive surgery. The ideal graft is one that provides a stable knee, which allows for early rehabilitation and return

to level of activity without causing significant morbidity from graft harvest. This study shows that, when carefully selected, nonirradiated fresh-frozen allografts are an acceptable alternative for use in a young active population. When the preferred characteristics are targeted, multistranded and from a female donor <50 years old or a male donor, the reinjury rates are significantly lower: 7.5% versus 30.2% in the nonpreferred group. This suggests that the characteristics of the donor and the morphology of the allograft are integral components to consider in ACL reconstructive surgery.

CONCLUSION

Fresh-frozen nonirradiated allografts may be an acceptable graft choice for ACL reconstruction in the young active population when the graft is carefully selected. The age and sex of the donor and the graft morphology significantly influence the rates of ACL graft rupture. Tendons from female donors aged ≥50 years should be avoided given the higher early rerupture rates as compared with donor tendons from male donors of any age and young female donors, and multiple-strand tendon allografts may be preferable to single-strand tendon allografts.

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