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Quantitative imaging of anterior cruciate ligament (ACL) graft demonstrates longitudinal compositional changes and relationships with clinical outcomes at 2 years after ACL reconstruction

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Abstract

$T_1\rho$ and T_2 magnetic resonance imaging (MRI) may allow for a noninvasive assessment of ligamentization after anterior cruciate ligament (ACL) reconstruction. We hypothesized that ACL graft $T_1\rho$ and T_2 relaxation times would decrease over time, that $T_1\rho$ and T_2 relaxation times would be inversely correlated with Knee Osteoarthritis Outcome Scores (KOOS), and that $T_1\rho$ and T_2 values would be lower for autograft relative to allograft reconstruction. Thirty-nine patients (age: 30.5 ± 8.2 years) were followed prospectively after ACL reconstruction with hamstring autograft (N = 27) or soft-tissue allograft (N = 12). Magnetic resonance (MR) imaging and KOOS surveys were completed at 6, 12, 24, and 36 months after surgery. ACL graft was segmented to define $T_1\rho$ and T_2 relaxation times. Relaxation times were compared between time points with ANOVA tests. Log-transformed autograft and allograft relaxation times were compared with the Student t tests. The relationship between KOOS and relaxation times at 24 months was investigated with Spearman's rank correlation. ACL graft $T_1\rho$ relaxation times were significantly higher at 6 months relative to 12 months ($P = .042$), 24 months ($P < .001$), and 36 months (P

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AUTHOR CONTRIBUTIONS

DL contributed to the design of the current study, overseeing the study, data analysis, data interpretation, and manuscript preparation. WX contributed to image processing/segmentation, data analysis, and manuscript preparation. AZ, CA, and BF contributed to patient recruitment, data interpretation, and manuscript preparation. XL and SM contributed to funding, initial cohort/study design, and data interpretation. CBM contributed to funding, initial cohort/study design, data interpretation, patient recruitment, current study design, data analysis, and manuscript preparation.

CONFLICT OF INTERESTS

The authors wish to disclose the following potential conflicts of interest: Drew Lansdown received fellowship research/education funding from Arthrex, Inc and Smith & Nephew, is a committee member for the Arthroscopy Association of North America, and has received hospitality support from Tornier/Wright Medical. Alan Zhang is a consultant for Stryker. Xiaojuan Li has received research funding from Ferring Pharmaceuticals. Sharmila Majumdar has received research funding from GE and Arthritis Foundation. C. Benjamin Ma is a consultant for Conmed Linvatec, Medacta, Histogenics, Stryker, and Tornier, and has received research support from Zimmer.

< .001). ACL graft T_2 relaxation times were significantly higher at 6 months relative to 12 months ($P = .036$), 24 months ($P < .001$), and 36 months ($P < .001$). $T_1\rho$ and T_2 relaxation times were significantly lower for autograft reconstruction vs allograft reconstruction at 24 months postreconstruction. Two-year KOOS Sports, Pain, and Symptoms were significantly inversely correlated with $T_1\rho$ and T_2 relaxation times. $T_1\rho$ and T_2 sequences may offer a noninvasive method for monitoring ACL graft maturation that correlates with patient-reported knee function after ACL reconstruction.

Keywords

ACL; clinical outcomes; diagnostic imaging; knee; ligament; proteoglycans; surgical repair

1 | INTRODUCTION

Injuries to the anterior cruciate ligament (ACL) occur in over 200 000 people annually in the United States. Surgical treatment involves reconstruction of the ACL with a tendon graft, either autograft, or allograft tissue. The structure and composition of these tendons; however, differ from that of the ligament, including higher proteoglycan content in ligaments and differences in collagen distribution.[1–3] Following surgical reconstruction, the tendon graft must, therefore, undergo a process of remodeling, known as "ligamentization," where the ACL graft becomes more structurally and biochemically similar to the native ACL.[4]

The process of ligamentization occurs in three general stages: early, remodeling, and maturation of the graft.[1] The early phase is marked by a decrease in cellularity and no revascularization.[5] During the remodeling, or proliferative phase, there is increased cellular activity and cellular density peak. Finally, the graft becomes more mechanically and biological similar to the native ligament in the final maturation stage through ongoing remodeling.[1,5] The time course of this progression is unpredictable and may take from 9 months to 2 years.[6–9] While the ACL graft is remodeling, the patient is undergoing a postoperative rehabilitation program. Progression of activities is based often on functional strength recovery, coordination, and time from surgery without a true understanding of graft healing or graft mechanical properties.[10]

The biochemical information obtained from quantitative magnetic resonance imaging sequences can reflect factors such as the proteoglycan content and collagen structure that are known to change through the course of ligamentization.[11–13] The $T_1\rho$ time course is directly related to proteoglycan content, and T_2 provides information on the structural alignment of collagen.[11,14] The role of $T_1\rho$ and T_2 mapping in monitoring graft changes after ACL reconstruction and how these metrics relate to patient outcomes remains unclear.

The purpose of this study was to evaluate the longitudinal progression of advanced quantitative imaging measurements of the ACL graft following reconstruction. We also sought to relate the graft measurements to patient-reported outcome (PRO) measures. We hypothesized that both $T_1\rho$ and T_2 values would decrease over time, reflecting an increase in proteoglycan content ($T_1\rho$) and overall improved organization of collagen structure (T_2). Additionally, we hypothesized that PROs would be significantly inversely correlated with

imaging-based graft characteristics at 2 years after ACL reconstruction, with higher Knee Osteoarthritis Outcome Scores (KOOS) correlated with lower $T_1\rho$ and T_2 values.

2 | METHODS

2.1 | Level of evidence: analytic, prospective cohort study; level 2

A prospective cohort of 39 patients (mean age: 30.5 ± 8.2 years; body mass index: 23.5 ± 2.5 kg/m^2) was recruited following acute ACL injury and prior to ACL reconstruction. The original purpose of study recruitment was to identify potential factors associated with posttraumatic arthritis after ACL injury.[15–17] Patients were included if they were between ages 15 and 50 years old, and sustained an isolated unilateral ACL injury. Exclusion criteria were a history of inflammatory arthritis, prior surgery on either knee, treatment with meniscus repair, or inability to obtain an magnetic resonance imaging (MRI). All procedures for this study were approved by our Institutional Review Board.

All patients underwent arthroscopic ACL reconstruction with a hamstring autograft (N = 27) or soft-tissue allograft (N = 12) after discussion of graft choice with their treating surgeon. Surgical reconstruction was performed by one of four fellowship-trained sports medicine orthopedic surgeons and with independent drilling of the femoral and tibial tunnels. Femoral fixation was achieved with suspensory fixation (N = 26: TightRope, Arthrex, Inc, Naples, FL and N = 1: EndoButton, Smith & Nephew, Andover, MA), and nonmetallic interference fixation was used on the tibial side (N = 27: Intra-Fix, Depuy Synthes, Raynham, MA). Postoperatively, patients were prescribed a standardized rehabilitation program. In general, patients were partial weight-bearing with crutches for 3 weeks and used a hinged knee brace for 6 weeks after surgery. Return to activities was based on functional strength recovery, with a return to running targeted at 4 to 5 months after surgery and return to sports at 9 to 12 months after surgery.

Postoperative magnetic resonance (MR) imaging was acquired at 6, 12, 24, and 36 months after surgery. Scans were completed on a 3T GE MR scanner (General Electric, Milwaukee, WI) with a dedicated eight-channel phased-array knee coil (Invivo, Orlando, FL). A high-resolution 3D fast spin-echo sequence (CUBE; repetition time/echo time = 1500/25 ms, the field of view = 16 cm, matrix = 384 × 384, slice thickness = 1 mm) was obtained in the sagittal plane. A 3D combined $T_1\rho/T_2$ MAPSS sequence was acquired in the sagittal plane (field of view = 14 cm, matrix = 256 × 128, slice thickness = 4 mm, views per segment = 64, time of recovery = 1.2; time of spin lock = 0/10/40/80 ms for $T_1\rho$; spin-lock frequency = 500 Hz; preparation echo time = 0/13.7/27.3/54.7 ms for T_2).[18]

At each imaging acquisition, patients completed the KOOS. This survey has been validated for use in patients after ACL injury and includes five domains: Sports, Symptoms, Quality of Life, Activities of Daily Living, and Pain.[19,20]

After the acquisition, images were transferred to a secure server for data processing. The CUBE images were semi-rigidly registered to the first echo of the $T_1\rho/T_2$ MAPSS sequence, and each individual echo was registered to the first echo. A pixel-by-pixel exponential fit was performed to determine the $T_1\rho$ and T_2 values.[18] Segmentation of the ACL graft was

performed manually on sagittal CUBE fat-saturated images (Figure 1). The intra-articular graft was segmented on multiple slices to create a volume of graft segmented for analysis. The segmentation was then transferred to the first echo of the $T_1\rho$ and T_2 images. The mean $T_1\rho$ and T_2 value for this defined region of interest was then determined with segmentations performed by a single author (WX) and all reviewed by a second author (DAL).

Statistical analyses were performed using Stata software (version 14, StataCorp, College Station, TX). Logarithmic transformation of $T_1\rho$ and T_2 values were analyzed after Shapiro-Wilk tests were used to confirm nonnormal distributions. The log-transformed $T_1\rho$ and T_2 were compared between multiple time points with analysis of variance tests and post hoc Bonferroni corrections of multiple comparisons. At each time point, the log-transformed $T_1\rho$ and T_2 values were compared between autograft and allograft reconstructions with unpaired the Student *t* test or Wilcoxon sign rank test as appropriate. Correlations between each KOOS subscore and $T_1\rho$ and T2 values at 2 years after surgery were tested with Spearman's rank correlation coefficients. Statistical significance was defined as $P < .05$.

3 | RESULTS

There was a significant difference in $T_1\rho$ relaxation times over time ($F = 9.6$; $P < .0001$). $T_1\rho$ relaxation times of the ACL graft were significantly higher at 6 months (36.7 ± 1.2 ms) relative to 12 months (33.2 ± 1.1 ms; $P = .042$), 24 months (30.9 ± 1.2 ms; $P < .001$) (Figure 2), and 36 months (30.7 ± 1.2 ms; $P < .001$) (Figure 3A). There were no other significant differences between other time points.

There was also a significant difference in T_2 relaxation times over time ($F = 11.0$; $P < .0001$). T_2 relaxation times of the ACL graft were significantly higher at 6 months (25.9 ± 1.2 ms) relative to 12 months (23.0 ± 1.2 ms; $P = .036$), 24 months (21.3 ± 1.2 ms; $P < .001$), and 36 months (20.6 ± 1.2 ms; $P < .001$) (Figure 3B). There was no significant difference between the other time points.

There were no significant differences between autograft and allograft groups for patient demographics or associated meniscus procedures (Table 1). The $T_1\rho$ relaxation times were significantly lower for autograft relative to allograft reconstructions at 24 months (29.6 ± 1.1 vs 33.7 ± 1.2 ms; $P = .019$) and trended toward being significantly lower for autograft relative to allograft reconstructions at 36 months (29.2 ± 1.2 vs 33.5 ± 1.2 ms; $P = .050$) (Figure 4A). The T_2 relaxation times were significantly lower in autograft relative to allograft reconstructions at 24 months (20.4 ± 1.2 vs 23.3 ± 1.2 ms; $P = .036$) and trended toward being significantly lower for autograft relative to allograft reconstructions at 36 months (19.5 ± 1.2 ms vs 22.6 ± 1.3 ms; $P = .060$) (Figure 4B). There were no significant differences between the $T_1\rho$ and T_2 values of autograft and allograft tissue at 6 months or 12 months.

The 2-year $T_1\rho$ and T_2 relaxation times showed multiple significant correlations with KOOS subscores (Table 2). A strong correlation was observed between the KOOS Sports subscore and T_2 relaxation times (Figure 5). Moderate correlations were observed between KOOS Sports subscore and $T_1\rho$ relaxation times and Pain subscores and $T_1\rho$ and T_2 relaxation

times. Weak correlations were observed between T2 relaxation times with KOOS Symptoms subscores and KOOS Quality of Life subscores.

4 | DISCUSSION

$T_1\rho$ and T_2 relaxation times of the ACL graft decreased significantly in longitudinal evaluation after ACL reconstruction. At 24 months, the $T_1\rho$ and T_2 values were significantly lower in autograft reconstructions relative to the allograft, while there was a trend toward lower $T_1\rho$ and T_2 values in autograft reconstruction at 36 months after reconstruction. These shorter relaxation times reflect higher proteoglycan content, which is inversely correlated with the $T_1\rho$ relaxation time, and more organized collagen structure, which is reflected in the T_2 relaxation time. Additionally, the 24 months postoperative imaging measures were significantly correlated with the KOOS subscores.

Biercevicz et al[14] utilized T_2* MRI to evaluate ACL reconstruction and ACL repair in a porcine model.[21] The biomechanical properties of the ACL, including yield load, stiffness, and maximum load, were correlated with the T_2* measurements. Those findings would suggest that the measurements observed in the current study reflect improving biomechanical properties of the graft between 6 and 24 months before reaching a relative steady-state. These noninvasive imaging sequences may, therefore, be able to identify a graft that is strong enough for athletic activity in a noninvasive manner.

We observed differences in the $T_1\rho$ and T_2 relaxation times between autograft and allograft tissue at 24 months with a trend toward differences at 36 months after surgery. Earlier in the recovery period (6 and 12 months), there were no observed differences between grafts. This may reflect greater biologic remodeling potential in the autograft reconstruction, similar to the observations from Muramatsu et al[22] in evaluating autograft and allograft reconstructions with contrast-enhanced MRI. Clinical studies have demonstrated that autograft tissue allows for a lower graft failure rate.[23,24] The observed differences may represent the progression over time of autograft tissue to stronger biomechanical construct relative to allograft tissue.

Importantly, the $T_1\rho$ and T_2 measurements appear to be clinically relevant, as the 2 years postoperative measurements were significantly correlated with multiple KOOS subscores. The strongest relationships were observed with the more functional subscores, especially the Sports subscore. The 2-year time point was chosen to evaluate clinical outcomes as functional recovery appears to take approximately 2 years following ACL reconstruction.[25] These observations are in line with previous reports from Biercevicz et al[26] that MRI signal intensity of the graft combined with graft volume was significantly associated with KOOS subscores. The observation of a relationship between graft composition and KOOS subscores suggests that a biomechanically and biochemically-superior graft is associated with improved clinical outcomes and offers a specific target for improving the outcomes of patients after ACL reconstruction. Future studies will clarify if these imaging sequences can distinguish a graft that is strong enough for advanced sporting activity.

The size of differences in relaxation times between groups is important to consider. We observed approximately 5 ms difference in $T_1\rho$ values and 3 to 4 ms difference in T_2 values

between autograft and allograft patients. The reproducibility of $T_1\rho$ and T_2 values for repeat scans has been reported as 1.2% to 2.7% for $T_1\rho$ and 3.1% to 4.0% for T_2.[27] The differences observed between autograft and allograft signal measurements in our study were greater than 10% for both measurements. The observed differences between groups are similar in magnitude to those reported for differences in the measurements of articular cartilage in patients with early osteoarthritis.[13,28] With early osteoarthritis; however, patients have increasing $T_1\rho$ and T_2 values as cartilage degeneration occurs, while we observed the opposite overall trend in our cohort with decreasing ACL graft $T_1\rho$ and T_2 values over time as graft maturation occurs. Finally, it is important to understand the magnitude of important differences in KOOS subscores for patients after ACL reconstruction. Previous studies have reported the minimal clinically important difference for the KOOS subscores in the setting of ACL reconstruction as ranging from 1 to 8 points.[29,30] Given that small differences in the KOOS score are clinically relevant, slight changes in $T_1\rho$ and T_2 measurements may be enough to meaningfully influence outcomes after ACL reconstruction. With these findings, the magnitude of differences in $T_1\rho$ and T_2 values and the relationship of those differences with KOOS subscores do seem to be both measurable and relevant in the clinical application of these findings.

This study should be interpreted with an understanding of its limitations. First, images were acquired in the sagittal plane and optimized primarily for cartilage imaging. Second, while there is an observed relationship between the 2-year KOOS subscores and the $T_1\rho$ and T_2 relaxation times, this study design does not allow for the determination of causation between these variables. This finding may reflect that a stronger graft allows for improved patient activity levels. Alternatively, the measurements of the graft may be a reflection of greater activity. Future studies will aim to clarify if and how these factors are causally linked. Third, we are not able to control for all potential variables that may influence postoperative outcomes. The autograft reconstruction group was slightly younger than the allograft group, though there was no statistically significantly difference in age, though the age may contribute to the observed changes. There was no statistically significant difference in the number of patients with partial meniscectomy, though again there may be potential differences between groups due to the small sample size. There were also unequal numbers of autograft and allograft reconstruction patients evaluated. Again, future larger studies on this topic will work to incorporate potential covariates that may influence the timing of graft maturation. Fourth, the echo times utilized were optimized for cartilage imaging rather than optimized for the reconstructed ACL, though the mean values of the ACL graft are within an appropriate range for the echo times of these scans. Future work can clarify the optimal sequence parameters for ACL graft evaluation. Finally, while the KOOS score is validated for application after ACL reconstruction, this PRO, like others, may still be subject to floor or ceiling effects leading to incomplete detection of findings in this group of patients.

5 | CONCLUSIONS

In conclusion, we observed significant changes over time in the graft $T_1\rho$ and T_2 relaxation times that are consistent with descriptions of in vivo ligamentization. Autograft ACLs showed a greater progression over time in comparison to allograft reconstructions. Finally, 2 years postoperative outcome scores were significantly associated with the ACL graft $T_1\rho$

and T_2 measurements. Quantitative MRI may allow for a noninvasive method to monitor ACL graft ligamentization after reconstruction and serve as a marker for patient-specific rehabilitation plans.

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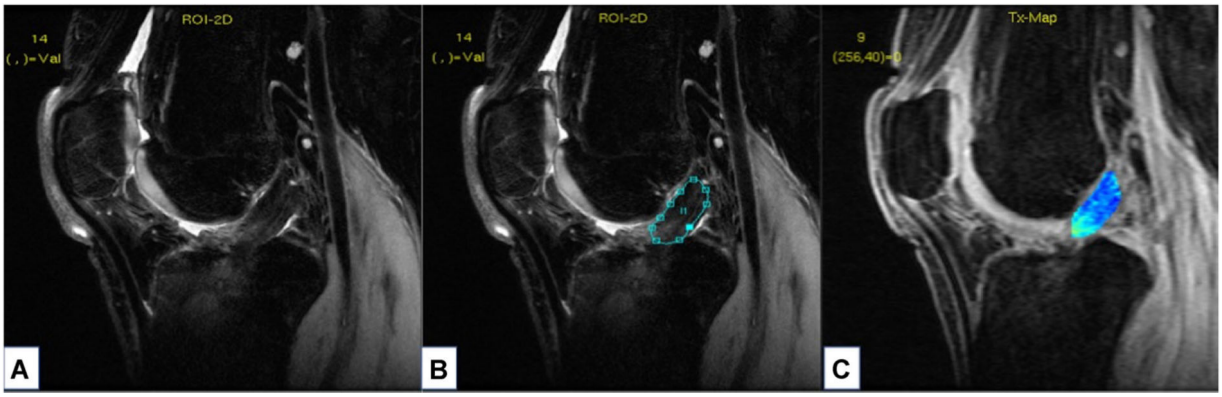

FIGURE 1.

The intra-articular portion of the reconstructed anterior cruciate ligament was identified on the (A) sagittal CUBE fast spin-echo images. The graft was (B) manually segmented and this was then (C) transferred to the $T_1\rho$ color map for quantification

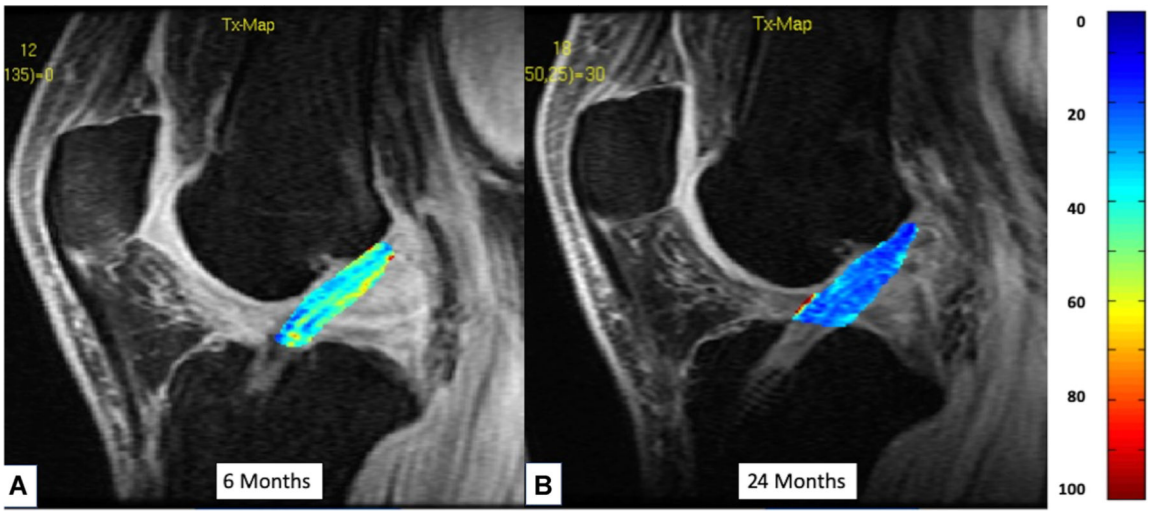

FIGURE 2.

The $T_1\rho$ relaxation time color map is displayed for the intra-articular ACL graft at (A) 6 months and (B) 24 months after ACL reconstruction with hamstring autograft. $T_1\rho$ values (milliseconds) correspond to the color bar. The ACL graft $T_1\rho$ relaxation time was 43.7 ms at 6 months and 23.9 ms at 24 months after surgery. ACL, anterior cruciate ligament

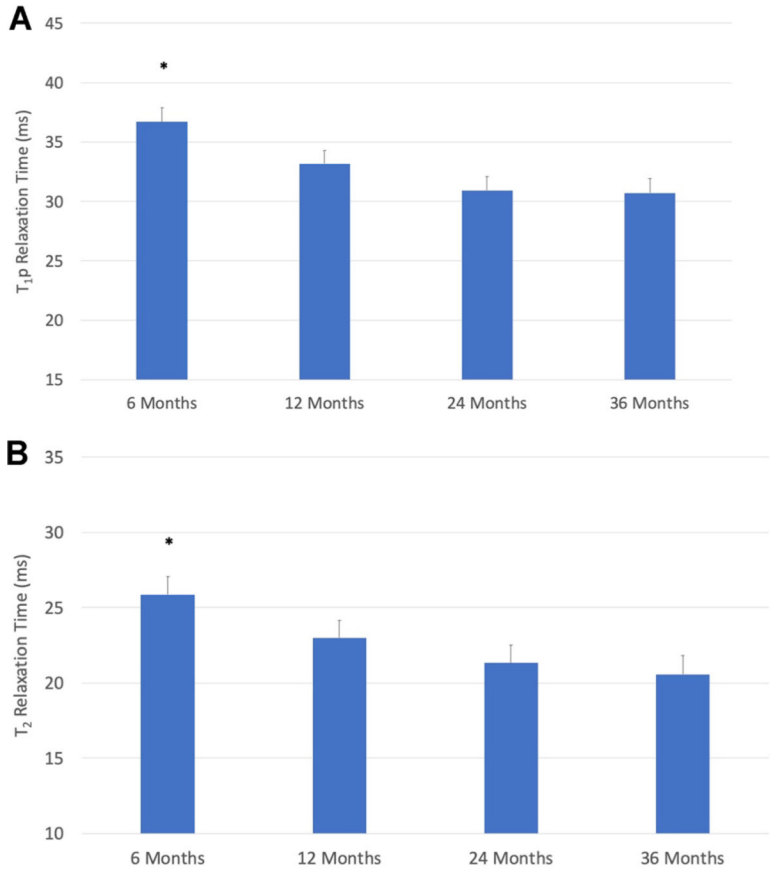

FIGURE 3.

The (A) mean $T_1\rho$ relaxation time and (B) mean T_2 relaxation time are shown for patients following ACL reconstruction at four postoperative time points. indicates $*P \leq .05$ relative to 12, 24, and 36 months. ACL, anterior cruciate ligament

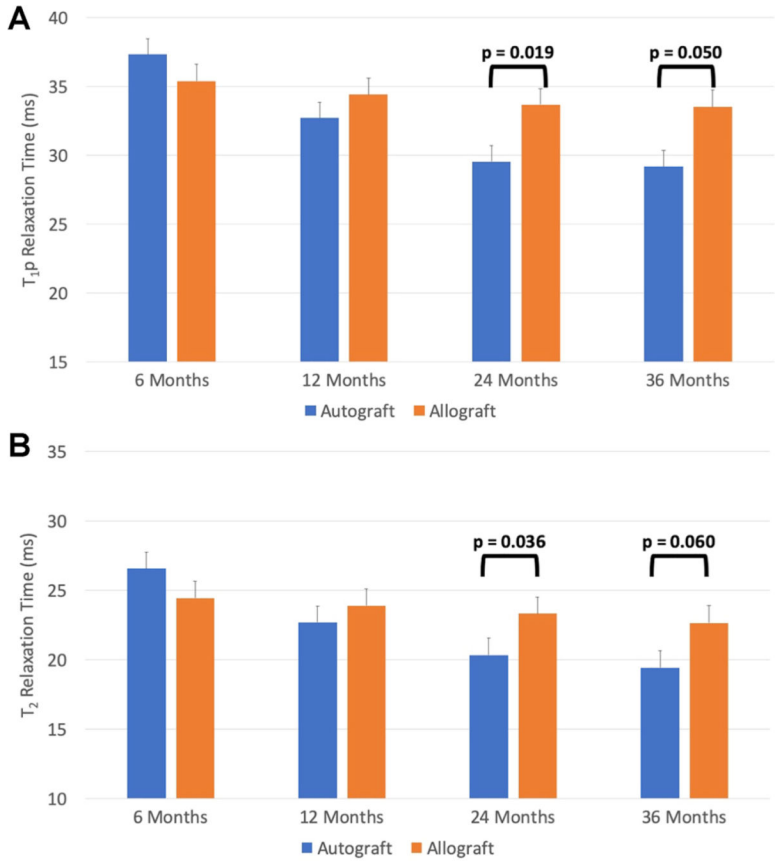

FIGURE 4.

The (A) mean $T_1\rho$ relaxation time and (B) mean T_2 relaxation time are shown between autograft (blue) and allograft (orange) ACL reconstruction at four postoperative time points. ACL, anterior cruciate ligament

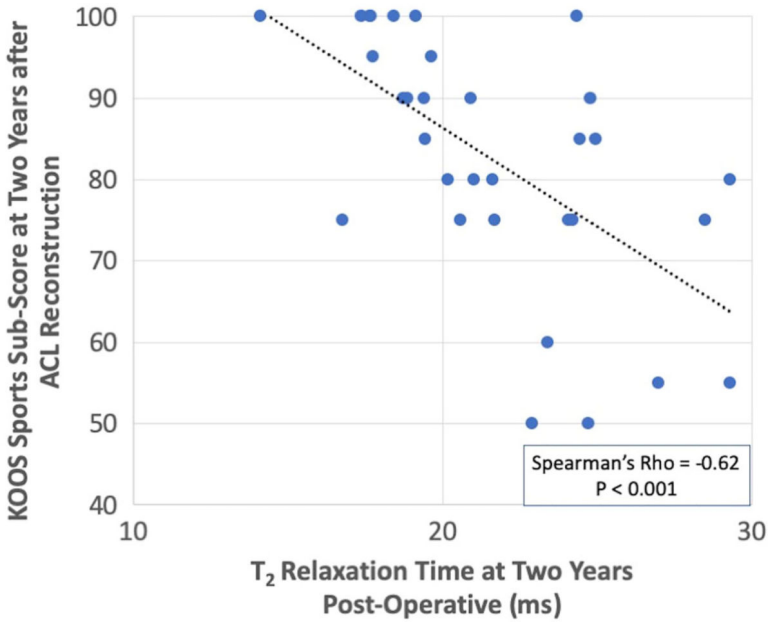

FIGURE 5.

A scatter plot demonstrates the relationship between T_2 relaxation times and the Knee Injury and Osteoarthritis Outcome Score (KOOS) Sports subscore is shown at 2 years after ACL reconstruction. ACL, anterior cruciate ligament

TABLE 1

Demographic comparisons between patients with autograft and allograft ACL reconstruction

	Autograft reconstruction (N = 27)	Allograft reconstruction (N = 12)	*P* value
Age (y)	29.5 ± 8.1	32.7 ± 8.3	.27
Body mass index, kg/m^2	23.6 ± 2.7	23.4 ± 2.1	.81
Sex			
Female	10	7	
Male	17	5	.30
Partial lateral meniscectomy	6	1	.40
Partial medial meniscectomy	1	0	1.0

Abbreviation: ACL, anterior cruciate ligament.

TABLE 2

Correlations between ACL graft T_2 and $T_1\rho$ measurements and KOOS subscores at 2 years after ACL reconstruction

		Two year Postperative KOOS subscores				
Imaging sequence		**Sports**	**Pain**	**Symptoms**	**Quality of life**	**ADLs**
T_2	Spearman ρ	**−0.62**	**−0.53**	**−0.39**	**−0.38**	−0.31
	P value	**<.001**	**.002**	**.03**	**.03**	.08
$T_1\rho$	Spearman ρ	**−0.58**	**−0.46**	−0.35	−0.31	−0.24
	P value	**<.001**	**.009**	.05	.09	.19

Abbreviations: ACL, anterior cruciate ligament; KOOS, Knee Osteoarthritis Outcome Score.

Bold values indicate $P < .05$.