Two-year postoperative MRI appearances of anterior cruciate ligament hamstrings autografts are not correlated with functional outcomes, anterior laxity, or patient age

Aims
MRI has been suggested as an objective method of assessing anterior cruciate ligament (ACL) graft “ligamentization” after reconstruction. It has been proposed that the MRI appearances could be used as an indicator of graft maturity and used as part of a return-to-sport assessment. The aim of this study was to evaluate the correlation between MRI graft signal and postoperative functional scores, anterior knee laxity, and patient age at operation.

Methods
A consecutive cohort of 149 patients who had undergone semitendinosus autograft ACL reconstruction, using femoral and tibial adjustable loop fixations, were evaluated retrospectively postoperatively at two years. All underwent MRI analysis of the ACL graft, performed using signal-to-noise quotient (SNQ) and the Howell score. Functional outcome scores (Lysholm, Tegner, International Knee Documentation Committee (IKDC) subjective, and IKDC objective) were obtained and all patients underwent instrumented side-to-side anterior laxity differential laxity testing.

Results
Two-year postoperative mean outcome scores were: Tegner 6.5 (2 to 10); Lysholm 89.8 (SD 10.4; 52 to 100); and IKDC subjective 86.8 (SD 11.8; 51 to 100). The objective IKDC score was 86% A (128 patients), 13% B (19 patients), and 1% C (two patients). Mean side-to-side anterior laxity difference (134 N force) was 0.6 mm (SD 1.8; -4.1 to 5.6). Mean graft SNQ was 2.0 (SD 3.5; -14 to 17). Graft Howell scores were I (61%, 91 patients), II (25%, 37 patients), III (13%, 19 patients), and IV (1%, two patients). There was no correlation between either Howell score or SNQ with instrumented anterior or Lysholm, Tegner, and IKDC scores, nor was any correlation found between patient age and ACL graft SNQ or Howell score.

Conclusion
The two-year postoperative MRI appearances of four-strand, semitendinosus ACL autografts (as measured by SNQ and Howell score) do not appear to have a relationship with postoperative functional scores, instrumented anterior laxity, or patient age at surgery. Other tools for analysis of graft maturity should be developed.

Cite this article: Bone Jt Open 2021;2-8:569–575.

Keywords: Reconstruction, MRI, Signal-to-Noise Quotient (SNQ), Anterior cruciate ligament (ACL)

Introduction
Following anterior cruciate ligament (ACL) reconstruction, hamstring autografts undergo postoperative structural transformation, described by Amiel et al as “ligamentization”. During this process of graft maturation, histological changes are visible for two to four years postoperatively with a phased remodelling process demonstrated histologically in both animal and human studies. There is general agreement that, following implantation, the ACL graft
undergoes a healing (or early) phase, a remodelling and proliferative phase, a maturation phase, and finally a quiescent phase, by which time the tendon eventually appears more similar to a ligament while never quite achieving an identical appearance to the native ACL.

Many factors including graft type, fixation method, and postoperative rehabilitation may influence the graft maturation process, potentially altering its biomechanical properties, histological appearances, and function. Cortical fixation allows a strong resistance but could create a widening of the tunnel due to micromotion by the “bungee cords effect” and “windshield wiper effect”. Having a tool to indicate graft maturity would be of great clinical utility, particularly in assisting decision-making with regard to allowing return to sports. MRI has been used to evaluate graft vascularity and has been proposed as a method for determining ACL graft maturity, yet there is little consensus. Changes in MRI appearance of the graft have been noted to follow a two-year course, similar to histological studies. This has led some authors to suggest that MRI graft signal can be correlated with mechanical properties and functional results, and to recommended MRI graft analysis before deciding on return to play. However, other studies have shown no relationship between the MRI appearances of an ACL graft and functional results.

We hypothesized that MRI graft signal in four-strand semitendinosus ACL autografts, at two-year postoperative follow-up, would correlate with functional outcome scores, measured anterior knee laxity, and with younger age at the time of surgery.

Methods

We identified a consecutive cohort of 154 patients who underwent ACL reconstruction with an ipsilateral, four-strand, semitendinosus (4ST) autograft using femoral and tibial suspensory fixation between July 2014 and May 2017. It was a single-site study and all patients were operated by the same surgeon (PC). Patients were excluded if they had concomitant lesions of the collateral ligaments or posterior cruciate ligament (PCL), a history of contralateral ACL injury or surgery (which might have affected side-to-side laxity analysis), and if they declined to participate. In order to assess whether patient age influenced the MRI appearances of the graft at two-year follow-up, patients were grouped according to age at surgery: < 25 years old, 25 to 34 years, 35 to 44 years, and > 45 years. All patients provided written informed consent for their participation in the study, which was approved by our institutional ethical review board (No. 12.2019.4).

Surgical procedure. The patients all underwent surgery under general anaesthesia with a surgical technique previously described in detail. All surgeries were done by the same senior surgeon (PC). Graft fixation was achieved using adjustable cortical devices: PullUp (SBM, France) on the femoral side and PullUp XL (SBM) on the tibial side. All patients followed a nonaggressive rehabilitation protocol with immediate weightbearing with crutches in a knee brace allowing full range of motion from day of the surgery.

Postoperative functional assessment. Lysholm, Tegner, and International Knee Documentation Committee (IKDC) subjective and objective scores were obtained at two years postoperatively. Side-to-side anterior laxity measurements were made using laximetry GnRB (GeNouRoB, France) at 134 N anterior drawer force.

MRI analysis. MRI scans were obtained for all 154 patients in the study cohort using a 1.5 T superconducting magnet (Signa; GE Healthcare, USA) with a dedicated surface coil. MRI slices were not analyzable for five patients due to movements of patients or artefacts. Five patients were excluded of the analysis as signal-to-noise quotient (SNQ) was not calculable. MRI protocol was the same for all patients. Imaging was confined to a thickness of 2 mm. Proton density-weighted images were acquired with the
standard spin echo technique (1,000 ms repetition time and 20 ms echo time). The proton density fat-saturated MRI sequences in sagittal planes were viewed and the slice showing the maximum cross-section of the graft was selected. MRI analysis of the graft was performed using the Howell score¹⁸ and SNQ, comparing signal in the ACL graft with the PCL signal and background signal¹¹,¹⁹ with a methodology similar to that used in previous studies.²⁰,²¹ All graft measurements were made using the RadiAnt DICOM Viewer 1.9.16 (Medixant, Poland). Radiological analysis was blinded to clinical results.

**Statistical analysis.** All calculations were made with Addinsoft 2020 (XLSTAT, France), with the level of statistical significance set at p < 0.05. Descriptive statistics were used to describe patient characteristics; continuous variables were expressed as mean and standard deviation (SD) and range and categorical data in terms of absolute numbers. A univariate analysis of variance (ANOVA) was used to analyze MRI graft appearance (SNQ and Howell score) and objective IKDC scores. The baseline characteristics of patients and demographic variables were compared between the different four groups of age, with the Pearson test for variables. The coefficient of determination (r²) was expressed. The strength of correlation p was defined as: 0.20 to 0.39 weak, 0.40 to 0.59 moderate, 0.60 to 0.79 strong, and 0.80 to 1.00 very strong. The chi-squared test or Fisher’s exact test were used for proportions.

To assess whether patient age influenced the MRI appearances of the graft at two-year follow-up, patients were grouped according to age at surgery: < 25 years old, 25 to 34 years, 35 to 44, and > 45 years. Differences in mean SNQ between groups were analyzed by ANOVA with Tukey post hoc analysis.

**Results**

Of the 149 patients, five were male and 54 female. The mean age of patients at surgery was 36.4 years (SD 12.5; 15 to 68): 38 patients aged < 25 years (25.5%), 34 patients aged 25 to 34 years old (22.8%), 39 patients aged 35 to 44 years old (26.2%), and 38 patients aged over 45 years old (25.5%).

Postoperative MRI scans at mean 25.7 months (SD 3.5 (24 to 55) follow-up showed a mean ACL graft SNQ of 2.0 (SD 3.5; -14 to 17) and Howell scores of I (n = 91; 61%), II (n = 37; 25%), III (n = 19; 13%), and IV (n = 2; 1%). ACL graft SNQ and Howell score correlated closely (p < 0.001, ANOVA) (Figure 1).

There was no correlation between patient age at surgery and two-year postoperative ACL graft SNQ (p = 0.554, Pearson test) or Howell score (p = 0.675, ANOVA) (Figure 2). ANOVA showed no statistically significant difference between mean ACL graft SNQ between age groups (Table I). Similarly there was no difference in the Howell scores between age categories (Table I).

At final follow-up, 85 patients (57%) had returned to sport at the same sporting level, 32 patients (22%) had returned to the same sport but at a lower level, 18 patients (12%) changed sports, and 14 patients (11%) did not return to sport. The postoperative IKDC objective scores were: 86% A (128 patients), 13% B (19 patients) and 1% C (2 patients). The mean Lysholm was 89.8 (SD 10.4), the mean subjective IKDC score was 86.8 (SD 11.8) and the mean Tegner score was 6.5 (2 to 10). The mean difference in side-to-side anterior laxity, as measured by GnRB laximetry was 0.6 mm (SD 1.8; -4.1 to 5.6). When comparing the functional outcome results by age category, we found no statistical difference. All coefficients of determination were considered weak. The value of r² depends on the range of the predictor variable. Here, the predictor variables move within a short range (Table I).

There was no correlation between ACL graft SNQ and Lysholm score (p = 0.720, Pearson test), subjective IKDC score (p = 0.492, Spearman test), objective IKDC score (p = 0.972, univariate ANOVA), Tegner score (p = 0.639, Pearson test) or anterior laxity (p = 0.163, Pearson test).

At final follow-up, nine patients had undergone a further surgical procedure: six arthroscopies for medial

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**Table I.** Functional scores and laximetry results for each age group.

| Variable | Total | < 25 yrs | 25 to 34 yrs | 35 to 44 yrs | > 45 yrs | p-value* | r²  |
|----------|-------|----------|--------------|--------------|----------|----------|-----|
| Patients, n | 149 | 38 | 34 | 39 | 38 | | |
| Mean IKDC subjective (range) | 86.8 (51 to 100) | 90.3 (69 to 100) | 87.7 (63 to 100) | 87.9 (51 to 100) | 81.3 (51 to 100) | 0.030 | 0.055 |
| IKDC objective n (%) | | | | | | | |
| A | 34 (89) | 29 (85) | 34 (87) | 31 (81) | | | |
| B | 3 (8) | 5 (15) | 5 (13) | 6 (16) | | | |
| C | 1 (3) | 0 | 0 | 1 (3) | | | |
| Mean Lysholm score (range) | 89.8 (52 to 100) | 90.4 (65 to 100) | 89.1 (52 to 100) | 91.2 (59 to 100) | 88.4 (59 to 100) | 0.883 | 0.01 |
| Mean Tenger score (range) | 6.5 (2 to 10) | 7.184 | 7.029 | 6.077 | 5.105 | < 0.001 | 0.212 |
| Mean SNQ (range) | 2.0 (-14 to 17) | 1.953 | 1.561 | 1.924 | 2.506 | 0.731 | 0.009 |
| Mean Howell grade (range) | 1.51 (1 to 4) | 1.605 | 1.529 | 1.462 | 1.526 | 0.869 | 0.005 |
| Mean delta GnRB at 134 N, mm (range) | 0.6 (-4.1 to 5.6) | 1.176 | 0.419 | 0.305 | 0.395 | 0.122 | 0.039 |

*Pearson test.

IKDC, Internation Knee Documentation Committee; SNQ, signal-to-noise quotient.
meniscus lesion (three meniscectomies for failure of medial meniscal repair and three meniscal repairs (one ramp and two lesions of the posterior horn); one tibial cyst with curettage and removal of the tibial device; one cyclops syndrome; and one infection with debridement and antibiotics at one month postoperatively.

**Discussion**

The main findings of this study were that the MRI appearances of 4ST ACL autografts at two-year postoperative follow-up do not correlate with objectively measured anterior laxity, functional outcome, or patient age.

Three phases of ACL graft ligamentization have been described corresponding to observed structural changes: an early (healing) phase, a remodelling phase, and a maturation phase, with some authors additionally reporting a forth “quiescent” phase. While there is agreement in the literature as to the sequence, the duration of these phases is disputed. Although Scheffler et al. and Uchio et al. reported that ACL graft maturation is largely complete at six months, others have described the process as continuing for between two to four years postoperatively, or even longer for complete graft maturation. We analyzed the maturation of the graft at two-year follow-up — the end of the maturation process but after the weakness period.

In a systematic literature review on return to play following ACL reconstruction, Barber-Westin and Noyes reported that in 32% of studies, time from surgery was the only criterion evaluated before patients were allowed to return to sport. However, it was felt that sole use of this criterion alone was insufficient, which would seem reasonable given the variability in reported graft maturation timescales. MRI has been proposed as a non-invasive alternative. Using routine MRI sequences, low signal in the graft, similar to that seen in the native PCL, is proposed to demonstrate advanced maturation. Conversely, hyperintense signal in the graft has been reported to correlate with poorer biomechanical properties and hypervascular/hypercellular reparative tissue. Uchio et al. found that high signal intensity in ACL grafts was correlated with delayed graft integration and instability. However the MRI analysis was performed using a simple classification of three levels of signal intensity: low signal, iso-signal, or high signal intensity. Lee et al. also found no correlation between the graft signal intensity and anterior laxity (measured with a KT-1000) in 98 patients at a follow-up of more than two years and Waltz et al. reported a variable correlation between MRI graft appearances and arthroscopic evaluation of the graft. Zhang et al. didn’t find any correlation between graft maturity and clinical scores.

The Howell score was described in 1991 in an effort to improve the quantification of signal intensity. Lower intensity graft signal was thought to relate to improved graft biomechanics. The SNQ was introduced in 1998 as a more accurate measure of ACL graft appearance and to decrease the influence of the background noise on graft signal intensity measurement. A higher SNQ was thought to signify poorer graft maturity and Weiler et al. reported a correlation between ACL graft histological changes and SNQ in sheep.

In our study, we evaluated grafts at two-year postoperative follow-up, when changes in anterior laxity occurring with ligamentization were likely to have stabilized and grafts were likely to be in an advanced stage of maturation. We found a close correlation between graft Howell score and SNQ values in line with those reported by previous studies (Table II). The wide variation in the range of mean SNQ values reported in the literature (between 0.1 and 0.4) may be explained by the different ACL grafts used in the studies and by differences in measurement technique, particularly in how point-of-interest was defined and the comparative reference tissue (PCL or quadriceps tendon). While the SNQ is a commonly used method of ACL graft analysis, it remains an imperfect tool, and the application of reconstruction filters may cause with deviations up to 140% relative to the reference method.

Many different factors influence MRI images including magnet strength, coil type, and type of sequence analyzed (turbo spin-echo, conventional spin-echo, or gradient-echo). It is possible that conventional MRI sequences may be insufficient to detect structural collagen changes due to non-specific echo times. Even MRI analysis of the graft with gadolinium enhancement has been shown to be problematic. Studies using this method have shown that MRI may have an insufficient threshold to detect vascularization, with some authors observing vascularization of the graft and others not. Improved ACL graft analysis may be possible with quantitative MRI sequences such as the recently proposed T1rho or UTE-T2* sequences.

At two-year postoperative follow-up, we found outcome scores similar to previous reported series in the literature. Mean anterior side-to-side anterior laxity was 0.6 mm (SD 1.8) comparing favourably with the results reported in Table II, although comparison is limited by the fact that we used GnRB at 134 N and most authors used KT-1000 (MedMetric, USA) or TELOS (Telos, Switzerland). We found no correlation between the SNQ and the functional and clinical scores and no correlation between either SNQ or Howell score and objectively measured anterior laxity. Hofbauer et al. also showed no correlation between SNQ and laximetry (using KT-1000) but this study was limited by a relatively small number of patients (n = 62) and the MRI analysis was performed at six months following surgery. Li et al. reported an association between graft SNQ and Tegner score but no correlation between SNQ and patient age. Their analysis was performed in 42 patients following hamstring autograft...
ACL reconstruction. Our findings, in a much larger series of patients, are similar to those of Li et al, 12 who also found no correlation between ACL graft SNQ and functional outcomes (IKDC, Lysholm, or Tegner scores) in 38 patients. Saupe et al 41 also reported similar conclusions, finding no correlation between ACL graft signal intensity, age, or IKDC score.

Our study with 149 patients is the largest study of the MRI appearances hamstring ACL autografts at two-year postoperative follow-up, giving strength to our results. However, this study has also some limitations. Although the SNQ is the most widely used measure of ACL graft appearance used in the literature, the index depends on the signal intensity described by Gohil et al 7 and is dependent on the characteristics of the MRI scanner, reconstruction algorithms, sequence type, and characteristics of the grayscale settings. Any modification of contrast to improve visualization of the MRI slice best showing the ACL can lead to alteration of the SNQ. A better tool for MRI analysis of the graft would be independent of signal intensity. Additionally, we analyzed ACL graft signal in 4ST autografts and our results may only be applicable to this type of graft. However, Rose et al 42 did not find differences between SNQ values for hamstrings and tibialis anterior tendon grafts at six-month postoperative follow-up, although their study used allografts. 42 Maturation of hamstring grafts has also been reported to be slower than bone-patellar tendon-bone graft maturation27 although at two years of postoperative follow-up, hamstring grafts were likely to be in a late stage of

| Year          | Author                | Patients, n | FU, yrs | Graft | Comparative | ROI shape | Dimensions ROI | Reference | Mean SNQ (SD) | Mean Lysholm score | Mean IKDC subjective | Mean Tegner score |
|---------------|-----------------------|-------------|---------|-------|-------------|------------|----------------|-----------|---------------|---------------------|----------------------|---------------------|
| 2020          | Our Study             | 148         | 2       | 4 ST  | Graft intra joint | Round     | 490 pixels PCL | 2 (3.5)  | 89.8         | 86.8               | 6.5                 |                     |
| 2020          | Zhang (China)         | 22          | 2       | ST-G  | Graft intra joint | Round     | 4 mm PCL | 4.5       | 90           | 89                 | N/R                 |                     |
| 2019          | Wen (China)           | 69          | 2       | ST-G  | Graft intra joint | Round     | 5 mm QT | 3.6 (1.1) | 95           | 89                 | N/R                 |                     |
| 2019          | Oshima (Australia)    | 98          | 2       | 4 ST  | Graft intra joint | Round     | 20 mm² PCL | 3.4 (2.1) | 92           | 86                 | 6.4                 |                     |
| 2019          | Li (China)            | 43          | 1       | ST-G  | Graft intra joint | Round     | 25.5 mm² QT | 19.4      | 59           | 68                 | 5                   |                     |
| 2019          | Jiang (China)         | 20          | 2.5     | ST-G  | Graft intra joint | Round     | 5 mm² PCL | 7.53      | 90           | 86                 | 6                   |                     |
| 2018          | Liu (China)           | 45          | 2       | ST-G  | Graft intra joint | Round     | 10 mm² QT | 15.3 (6.3) | 88.9         | 88.4               | 5.9                 |                     |
| 2018          | Lee (Korea)           | 100         | 3       | 4 ST  | Graft intra joint | Round     | 3 mm PCL | 3.3 (0.4) | 92           | 91                 | 6                   |                     |
| 2018          | Kim (Korea)           | 111         | 2       | Double bundle | Graft intra joint | Round     | 3 mm QT | 14.01 (12.6) | 91.5         | 85.9               | 6                   |                     |
| 2018          | Hofbauer (Austria)    | 62          | 6 months | ST-G | Graft intra joint | Round     | 19 mm² QT | 0.1       | N/R          | N/R                 | N/R                 |                     |
| 2018          | Chen (China)          | 48          | 1       | ST-G  | Allograft vs Autograft | Round     | 25.5 mm² PCL | 25       | 39           | 60                 | 5                   |                     |
| 2017          | Cavaignac             | 62          | 1       | 4 ST  | 4ST vs Hamstrings | Graft intra joint | Round     | 5 mm² PCL | 5.9 (3.7) | 92           | 89                 | 5.7                 |                     |
| 2017          | Toshio (UPMC)         | 24          | 2       | QT    | Graft intra joint | Round     | 20 mm² PCL | 1.5       | N/R          | N/R                 | N/R                 |                     |
| 2017          | Rose (USA)            | 32          | 6 months | 4 ST  | Allograft vs Autograft | Round     | 2.5 mm QT | 2.56 (2.41) | 11.6         | 24.4               | 2.8                 |                     |
| 2017          | Ahn (Korea)           | 81          | 6 months | 4 ST  | Graft intra joint | Round     | 3.3 mm QT | 3.1 (2.44) | 91.52         | 82.4               | N/R                 |                     |
| 2016          | Lee (Korea)           | 98          | 1.5     | ST-G  | Remnant vs no Remnant | Graft intra joint | Round     | 2.5 mm QT | 3.55 (1.47) | N/R          | N/R                 | N/R                 |                     |
| 2015          | Ma (UPMC)             | 26          | 6 months | QT cs HS | Graft intra joint | Round     | 5 mm PCL | 2.4 (0.5) | N/R          | N/R                 | N/R                 |                     |
| 2014          | Li (China)            | 104         | 1.5     | ST-G  | Allograft vs Autograft | Graft intra joint | Round     | 10 mm² QT | 9.2 (9.9) | N/R          | N/R                 | N/R                 |                     |
| 2013          | Tanaka (Japan)        | 83          | 6 months | ST-G | SB vs DB vs TB | Graft intra joint | Round     | * PCL     | 4.8 (2.2) | N/R          | N/R                 | N/R                 |                     |
| 2012          | Li (China)            | 52          | 2       | ST-G  | Allograft vs Autograft | Graft intra joint | Round     | 5 mm² QT | 3.56 (4.62) | 94.3         | 93.3               | N/R                 |                     |
| 2008          | Muramatsu (Japan)     | 44          | 2       | BTB   | Allograft vs Autograft | Graft intra joint | Round     | 11.66 mm² QT | 2        | N/R          | N/R                 | N/R                 |                     |
| 2001          | Vogl (Germany)        | 68          | 2       | Graft intra joint | Round     | 4 mm QT | 3         | N/R          | N/R          | N/R                 |                     |                     |
| 1997          | Stockle (Germany)     | 20          | 2       | †      | † TR   | †      | 1         | N/R          | N/R          | N/R                 |                     |                     |

*Non-defined zone. †With Tukey post hoc analysis as no hypothesis about the relationship between these groups was necessary before comparison. BTB, bone-patellar tendon-bone; DB, double bundle; FU, follow-up; G, gracilis; HS, hamstrings; QT, quadriceps tendon; ROI, region of interest; SB, single bundle; SD, standard deviation; SNQ, signal-to-noise quotient; ST, semitendinosus; TB, triple bundle.
maturation. Finally, we analyzed adjusted cortical fixation and MRI aspect of the graft could compare different mode of graft fixation.

In this large series of 4ST autograft ACL reconstructions with MRI follow-up at two years we did not find a correlation between the MRI appearances of the ACL graft with anterior laxity, functional outcome, or patient age.

**Take home message**

- Using signal-to-noise analysis of the graft of anterior cruciate ligament at two-years postoperatively, MRI appearances of hamstrings autografts are not correlated with functional outcomes, anterior laxity, or patient age.

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**Funding statement:**
This manuscript has been self-funded for open access publication. The author or one or more of the authors have received or will receive benefits for personal or professional use from a commercial party related indirectly to the subject of this article.

**ICMJE COI statement:**
- N. Bouguennec is a consultant for SBM, N. Graveleau is a consultant for SBM, and P. Colombet has received royalties from SBM, all of which is unrelated to this article.

**Ethical review statement:**
All patients provided written informed consent for their participation in this study, which was approved by the institutional ethical review board (No. 12.2019.4).

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