Assessment of Graft Maturity After Anterior Cruciate Ligament Reconstruction Using Autografts: A Systematic Review of Biopsy and Magnetic Resonance Imaging studies

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Purpose: The purpose of this investigation was to evaluate systematically the literature concerning biopsy, MRI signal to noise quotient (SNQ) and clinical outcomes in graft-maturity assessment after autograft anterior cruciate ligament reconstruction (ACLR) and their possible relationships. **Methods:** The systematic review was reported and conducted according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines. Studies through May 2019 evaluating methods of intra-articular ACL autograft maturity assessment were considered for inclusion. Eligible methods were histologic studies of biopsy specimens and conventional MRI studies reporting serial SNQ and/or correlation with clinical parameters. **Results:** Ten biopsy studies and 13 imaging studies, with a total of 706 patients, met the inclusion criteria. Biopsy studies show that graft remodeling undergoes an early healing phase, a phase of remodeling or proliferation and a ligamentization phase as an ongoing process even 1 year after surgery. Imaging studies showed an initial increase in SNQ, peaking at approximately 6 months, followed by a gradual decrease over time. There is no evident correlation between graft SNQ and knee stability outcome scores at the short- and long-term follow-up after ACLR. **Conclusions:** The remodeling of the graft is an ongoing process even 1 year after ACLR, based on human biopsy studies. MRI SNQ peaked at approximately 6 months, followed by a gradual decrease over time. Heterogeneity of the MRI methods and technical restrictions used in the current literature limit prediction of graft maturity and clinical and functional outcome measures by means of MRI graft SNQ after ACLR. **Level of evidence:** Level IV, systematic review, including level III and IV studies.

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Anterior cruciate ligament reconstruction (ACLR) has become a common procedure in the field of orthopedic sports medicine to restore stability in the knee and promote return to sport after rupture of the anterior cruciate ligament.¹ After surgery, the graft tissue changes from tendinous to ligamentous-appearing in the new intra-articular environment, a process also called remodeling.² Animal and human in vitro and in vivo research has demonstrated 3 characteristic stages of graft healing after ACLR: an early healing phase, followed by a phase of proliferation and, finally, a maturation or ligamentization phase.³⁻⁵ During these phases of healing, changes in cellularity, vascularity and extracellular matrix transform graft characteristics into properties of the intact ACL.^{6,7} However, full restoration of the biologic properties is not achieved.^{8,9}

Optimal timing for return to sport after ACLR remains an on-going debate.^{10,11} To prevent graft reinjury, the

safe return to sport is of great concern for both patients and physicians.^{12,13} Knee stability and patient-reported outcome measures (PROMs) have traditionally been used to evaluate the success of ACLR and timing of return to sport.^{10,14} However, these outcome measures may lack the sensitivity to determine graft maturity.^{11,15} Histologic analysis of biopsy graft specimens during second-look arthroscopy is considered the gold standard to determine graft maturity. However, this method is invasive and, therefore, not ideal for clinical follow-up.⁴ Magnetic resonance imaging (MRI) is noninvasive and potentially suitable to assess graft maturity longitudinally in vivo and guide the time of return to sport.^{15,16} Previous animal studies already demonstrated that larger graft volume and lower graft-signal intensity are correlated with higher strength and superior biomechanical properties of the reconstructed ACL.¹⁷⁻¹⁹ Likewise, human studies have investigated MRI signal-to-noise quotient (SNQ) and its relation to graft maturity.^{13,20-26}

Previous systematic reviews of remodeling focused mainly on human and animal biopsy studies³⁻⁵ and on graft-maturity assessment using MRI.²⁷ The purpose of this investigation was to evaluate systematically the current literature concerning biopsy, MRI SNQ and clinical outcomes in graft maturity assessment after autograft ACLR, and their possible relationships. Our hypothesis is that graft SNQ follows a clear pattern over time and resembles histologic remodeling stages and that a correlation with clinical parameters can be found.

Methods

Protocol and Registration

The systematic review was reported and conducted according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines.²⁸ Study protocol was registered with International Prospective Register of Systematic Reviews (PROSPERO), ID CRD42018094585.

Eligibility criteria

Studies reporting methods of intra-articular ACL graft maturity assessment, at any time after primary ligament reconstruction surgery using autografts, were considered for inclusion. Eligible methods were histologic studies of biopsy specimens and conventional MRI studies reporting serial signal intensity values and/or correlation of MRI signal intensity with clinical parameters such as knee stability scores and PROMs. Signal intensity had to be normalized to surrounding tissue and/or background noise such as the SNQ or signal-to-noise ratio (SNR). The following exclusion criteria were used: case reports, advanced MRI techniques (e.g., diffusion tensor imaging, angiography),

focus on tunnel healing, allografts-only studies, patients younger than 18 years of age, cadaver and animal studies, as well as secondary reconstructions. Language was limited to English, Dutch, German, French, and Portuguese.

Information Sources and Search

An experienced librarian performed the systematic search, until May 15, 2019, in Embase, MEDLINE ovid, Cochrane Central, Web-of-Science, Scopus, Cinahl EBSCOhost, Sportdiscus EBSCOhost, and Google Scholar. The search strategy employed included the following key terms: "knee ligament reconstruction," "anterior cruciate ligament reconstruction," "autograft," "bone patellar tendon bone graft," "hamstring tendon," "healing," and "remodeling."

Study Selection

Titles and abstracts of the search results were assessed for eligibility by 3 reviewers (B.v.G., D.M.J., R.P.A.J.). The same reviewers also assessed full texts of this selection for final inclusion. Any disagreement among the reviewers was resolved by discussion, and another reviewer could be consulted in case of nonconsent. All references of the included full texts were manually searched for potentially missed eligible articles.

Data Extraction

Data were extracted by 1 and verified by another author (B.v.G., D.M.J.). General study and patients' characteristics were extracted for each included study. Furthermore, for the biopsy studies, extracted data consisted of reported healing stages and examined tissue aspects (e.g., cellular aspects, vascularity, extracellular matrix). Data of interest from imaging studies consisted of MRI technique (e.g., magnet strength [Tesla], coil type, sequence, acquisition, slice/voxel size, field of view, and SNQ details). Extracted SNQ value was always selected from the autograft midportion and nonintervention group (if applicable). In addition, outcome and corresponding statistical measures (e.g., R-squared and correlation coefficients) describing the relationship between image and clinical/functional parameters were extracted.

Quality Assessment

Two reviewers (B.v.G., M.C.v.d.S.) independently assessed the individual studies for risk of bias. The assessment was based on the Cochrane Collaboration tool for assessing risk of bias.²⁹ Risk of bias and assessed types of bias are presented in Table 1. Items could be scored as low risk (+), high risk (−), unknown/not reported (?), or not applicable (NA). The assessments by the 2 independent reviewers were compared and discussed for final consensus. Another reviewer could be consulted in case of nonconsent.

Table 1. Risk of Bias in Included Studies

	Selection Bias 1 (Patient selection)	Selection Bias 2 (Patient characteristics)	Performance Bias	Detection Bias	Attrition Bias	Reporting Bias
Biopsy studies						
Abe (1993)	?	+	+	?	NA	+
Cho (2004)	?	+	+	?	+	+
Dong (2005)	+	+	+	+	NA	?
Falconiero (1998)	?	+	+	+	-	?
Janssen (2011)	+	+	+	?	NA	+
Marumo (2005)	+	+	+	?	+	+
Rougraff (1993)	-	?	+	?	NA	+
Sanchez (2010)	+	+	+	+	+	?
Zaffagnini (2007)	?	+	+	?	NA	+
Zaffagnini (2010)	?	+	+	?	NA	+
Imaging studies (serial SNQ)						
Chen (2018)	?	+	+	+	+	+
Gohil (2007)	+	+	+	+	+	+
Fukuda (2019)	+	+	+	?	+	+
Lee (2016)	-	+	+	+	+	+
Liu (2019)	+	+	+	+	+	+
Muramatsu (2008)	?	+	+	?	+	+
Stockle (1998)	?	+	+	+	NA	+
Tashiro (2017)	?	+	+	?	+	+
Vogl (2001)	-	+	+	?	NA	+
Imaging studies (clinical)						
Biercevicz (2015)	?	+	+	+	?	+
Hofbauer (2019)	+	?	+	?	NA	+
Li (2014)	?	+	+	?	+	?
Li (2017)	?	+	+	+	+	+

NA, not applicable; SNQ, signal to noise quotient; +, low risk of bias; -, high risk of bias; ?, unknown/not reported.

Results

Fig 1 shows the flowchart and details of the systematic search. Table 2 shows the characteristics of 10 included human biopsy studies with a total of 316 grafts.^{6-9,30-35} The majority of included cases were clinically stable after reconstruction. However, none of the included studies compared histologic findings to clinical or functional outcomes. Of the imaging studies, 10 reported serial MRI measurements using SNQ.^{13,20-24,26,36-38} Table 3 shows the study characteristics and MRI parameters of included imaging studies reporting serial SNQ; there was a total of 479 patients. A total of 4 imaging studies reporting on the relationship with clinical parameters could be selected; there was a total of 227 patients (Table 4).^{13,15,39,40}

Most included studies contained risk of bias (see Table 1 for the quality assessment of included studies), especially risk of selection bias due to the number of retrospective studies and case series. No study showed risk of performance bias. Furthermore, no biopsy study reached a level of evidence higher than 3 (Table 2). For the imaging/clinical studies, the highest level of evidence was 2 (Tables 3 and 4).

The Results section covers the following subjects: (1) histologic appearance of human hamstring tendon (HT) and bone patellar tendon bone (BPTB) autografts; (2) postoperative changes in MRI graft SNQ over time; and

(3) relation of imaging parameters with clinical and functional outcomes.

Histologic appearance of human autografts: summary of relevant findings of included studies

Fig 2 shows the ligamentization stages as published by Pauzenberger et al.,⁵ which was adapted from Claes et al.⁴ The studies show that the postoperative healing process of hamstring and BPTB autografts evolve in a typical progression through 3 distinguishable remodeling phases: an early healing phase, a phase of remodeling and a ligamentization phase.^{6-9,30} Despite variations in the reported duration of remodeling phases, included studies suggest a delayed progression in hamstring grafts compared to BPTB grafts.⁵ In the absence of new studies reporting results according to this timescale, this systematic review summarizes overall relevant histologic findings over time after ACLR, as grouped by cellular aspects, vascularity and extracellular matrix as reported in the included studies.^{6-9,30-35}

Cellular aspects

Cells were predominantly ovoid for the first 12 months after ACLR.^{8,30} Morphology changed to more spindle-shaped and linear cells between 13 and 24 months after reconstruction.^{8,30} After 3 years, cell shape was predominantly narrow and long.^{7,8}

Fig 1. Study flow chart.

Total cell numbers were significantly increased compared to the normal ACL during the first year after ACLR for both HT and BPTB grafts.⁶⁻⁹ Abe et al.⁹

showed the presence of so-called metabolically active fibroblasts in their ultrastructure evaluation of BPTB graft biopsies at 6 months and 1 year postoperatively,

Table 2. Characteristics of Biopsy Studies

	Abe (1993)	Cho (2004)	Dong (2015)	Falconiero (1998)	Janssen (2011)	Marumo (2005)	Rougraff (1993)	Sanchez (2010)	Zaffagnini (2007)	Zaffagnini (2010)
Study design	Case series	Case-control	Case-control	Case-control	Case-control	Case-control	Case series	Case-control	Case-control	Cross-sectional study
Level of evidence	4	3	3	3	3	3	4	3	3	3
N	21	25	52	48	67	50	23	37	10	8
Female	15	5 vs. 7	16	15	22	19	3	11	0	0
Mean age (range)	14-36	26 (18-29) vs. 25 (15-39)	29.0/29.6	26.1 (16-35)	Group 1 = 31.1; 2 = 27.7; 3 = 29.0	28.5 (15.1-49.0) vs 24.5 (13.3-44.0)	Not mentioned	28 (18-48)	25 (18-32)	26 (19-31)
Follow-up period	6 wks-15 mo	16 (7-27) vs. 20 (9-39) mo	13-62 mo	3 mo-10 yrs	6-117 mo	11-13 mo	3 wks-6.5 yrs	6-24 mo	6, 12, 24, 48, 120 mo	12, 24, 48, 120 mo
Independent examiner	Not mentioned	Not mentioned	2	2	Not mentioned	Not mentioned	Not mentioned	3 (blinded)	Not mentioned	Not mentioned
Graft type	BPTB	10 DB 2-strand (ST) vs 15 SB triple/quadruple (ST+/-GT)	DB 8-strand ST + GT	35 BPTB, 8 HS	Quadruple HS	30 BPTB, 20 ST+GT	BPTB	HS	BPTB	Quadruple ST+GT
Clinical outcome	Stable	Not mentioned	Stable (clinical failure excluded)	2 grade III/IV, 5 grade 2, and 41 grade I Lachman	Stable	Stable	Stable	Stable	KT 2000 <3 mm in 7 cases, 3-5 mm in 3 cases	KT 2000 was <3 mm in 6 cases, 3-5 mm in 2 cases
Reason for reoperation	HR	HR	HR	New symptoms	HR	HR	New pathology, HR, voluntary	New pathology, HR	Menisectomy or HR	Menisectomy or HR
Biopsy site	Central superficial	Central superficial	Central (midstrand)	Central superficial + deep	Central superficial	Most central part	Central superficial	Directly under femoral condyle	Most central part	Most central part
Analysis technique	LM + EM	EM	LM + EM	LM	Immunostaining + LM	LM, HPLC	LM	LM	EM	EM
Tissue aspects	V + C + F + Col	Col	C + V + Col	C + V + Col	V + C + F + Col	Col	C + V + Col	C + V	Col	Col

BPTB, bone-patellar tendon-bone; C, cellularity; Col, collagen; DB, double bundle; EM, electron microscopy; F, fibroblasts; GT, gracilis tendon; HR, hardware removal; HPLC, high-performance liquid chromatography; HS, hamstring; LM, light microscopy; PRGF, platelet rich growth factor; SB, single bundle; ST, semitendinosus tendon; V, vascularity.

Table 3. Study Characteristics of Imaging Studies With Serial SNQ Measurements

	Chen (2018)	Gohil (2007)	Fukuda (2019)	Lee (2016)	Liu (2019)	Muramatsu (2008)	Stockle (1998)	Tashiro (2017)	Vogl (2001)	Li (2017)*
Study design	Prospective cohort	Randomised prospective study	Case series	Retrospective cohort	Randomized controlled trial	Retrospective comparative study	Case series	Prospective cohort	Case series	Prospective cohort
Level of evidence	3	2	4	3	2	3	4	3	4	3
N	48	49	45	98	45	44	20	24	68	38
Female	Not mentioned	22	27	12	12	8	8	7	33	0
Mean age (SD/range)	30 ($\pm$ 6)/29 ($\pm$ 6)	35.5 (21-50)/30.5 (15-59)	27 ($\pm$ 8.5)	30.1/30.4	31.5/29.4	26.1 ($\pm$ 1.6)/26.5 ($\pm$ 1.2)	30 (17-59)	20 ($\pm$ 4)	36 (17-59)	30.8 ($\pm$ 5.9)/29.5 ($\pm$ 5.0)
Graft type [†]	ST + GT autograft, allograft	Double-loop ST + GT autograft	Double bundle ST/GT autograft	4-strand ST + GT autograft	4-strand ST + GT autograft	BPTB autograft + allograft	BPTB autograft	Quadriceps tendon autograft	BPTB autograft	4-strand ST/GT autograft, tibialis anterior allograft
MRI follow-up	3, 6, 12 mo	2, 6, 12 mo	3 wks, 3, 6, 9, 12, 18, 24, 36, 48, 50 mo	4 periodes, up to 18 mo	3, 6, 12, 24 mo	1, 4, 6, 12 mo	2, 12, 24, 52, 102 wks	6, 24 mo	2, 12, 52, 76, 104 wks	3, 6, 12 mo
Magnet strength (Tesla)	3.0	1.5	0.3	1.5	3.0	1.0	1.5	3.0	1.5	3.0
Coil type	Not mentioned	Knee coil	Not mentioned	Extremity coil	Not mentioned	Not mentioned	Knee coil	Not mentioned	Extremity coil	Not mentioned
Sequence	PD FS	PD FSE	PD SE	PD	FD FS	T1 Gd-DTPA	T1/T2 SE +T1 Gd-DTPA	DESS, short TE T2	PD SE T1/T2, T1 SE/FS Gd-DTPA	PD FS
Acquisition parameters (TR/TE) (ms)	Not mentioned	3000 / 30	1800 / 20	3000-4000 / 17-18	3000/28	500/17	NA	16.3/4.7-2700/10.6	800/5 500/15	3000/28
MRI slice thickness (mm)	Not mentioned	2	4	Not mentioned	3	Not mentioned	Not mentioned	3	3	3
MRI voxel size (mm)	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	0.45 x 0.45 x 0.70	Not mentioned	Not mentioned
Field of view (mm)	Not mentioned	Not mentioned	170	Not mentioned	150	Not mentioned	Not mentioned	Not mentioned	200/400	150
SNQ calculation	(SI graft-SI PCL)/SI background	SI graft/SI background	(SI graft-SI PCL)/SI background	(SI graft-SI quadriceps tendon)/SI background	(SI graft-SI quadriceps tendon)/SI background	(SI graft-SI quadriceps tendon)/SI background	(SI graft-SI patellar tendon)/SI background	(SI graft-SI PCL)/SI background	(SI graft-SI quadriceps tendon)/SI background	(SI graft-SI quadriceps tendon)/SI background
SNQ zones	4	4	1 site each bundle	3	3	1	3	3	3	3

BPTB, bone-patellar tendon-bone; DB, double bundle; DESS, double-echo steady state; FS, fat saturated; FSE, fast spin echo; Gd-dtpa, gadolinium diethylenetriamine penta-acetic acid; HS, hamstring; MRI, magnetic resonance imaging; PCL, posterior cruciate ligament; PD, proton density; SI, signal intensity; SNQ, signal to noise quotient; ST, semitendinosus tendon; TR, time-to-repetition.

*Same study as mentioned in Table 4.

[†]Only autograft data was used in the analyses.

Table 4. Study Characteristics of Imaging/Clinical Studies

	Biercevicz (2015)	Hofbauer (2019)	Li (2014)	Li (2017)*
Study design	Subset of ongoing RCT	Prospective comparative study	Case series	Prospective cohort
Level of evidence	3	2	4	3
N	23 (17 after 5 yrs)	124 (62 controls)	104	38
Female	13	Not mentioned	0	0
Mean age (SD/ range)	23(± 9)	Not mentioned	29.5 (± 6.3)	30.8 (± 5.9)/29.5 (± 5.0)
Graft type/origin [†]	BPTB (17/11), HS (6/5) autograft @ 3/5 yrs	4-strand ST/GT autograft	4-strand ST/GT autograft, tibialis anterior allograft	4-strand ST/GT autograft, tibialis anterior allograft
Clinical examination	AP knee laxity, 1- legged hop test	KT-1000	-	Anterior drawer, Lachman, pivot-shift, ATT
PROM	KOOS	-	Tegner activity score	IKDC, Lysholm Knee score, Tegner activity score
MRI/clinical follow- up	3 + 5 yrs	6 mo	30.7 ± 15.5 mo	3-6-12 mo
Magnet strength (Tesla)	3.0	1.5	3.0	3.0
Coil	Knee coil	Knee coil	Not mentioned	Not mentioned
Sequence	3D T1 flash	PD/T2 FS	PD FS	PD FS
Acquisition parameters (TR/ TE) (ms)	20/7.6	5000/102	3000 / 28	3000 / 28
MRI slice thickness (mm)	1.5	2.5	3	3
MRI voxel size (mm)	Not mentioned	Not mentioned	Not mentioned	Not mentioned
Field of view (mm)	160	160	150	150
SNQ/SNR	SI graft/SI bone	(SI graft-SI PCL)/SI background	(SI graft-SI quadriceps tendon)/SI background	(SI graft-SI quadriceps tendon)/SI background
SNQ/SNR zones	Entire graft median	3	Average of 3	3

ATT, anterior tibial translation; BPTB, bone-patellar tendon-bone; FS, fat saturated; GT, gracilis tendon; IKDC, international knee documentation committee score; KOOS, knee injury and osteoarthritis outcome score; MRI, magnetic resonance imaging; NA, not available; PCL, posterior cruciate ligament; PD, proton density; PROM, patient reported outcome measure; RCT, randomized controlled trial; SI, signal intensity; SNQ, signal to noise quotient; SNR, signal to noise ratio; ST, semitendinosus tendon; TR, time-to-repetition.

*Same study as mentioned in Table 3.

[†]Only autograft data was used in the analyses.

indicating ongoing remodeling. Janssen et al.⁸ found a strong increase in myofibroblast density from 13 up to 24 months after ACLR in HT grafts. This indicated an active remodeling process for HS grafts even 1 to 2 years after surgery.⁸

Dong et al.,³³ in their biopsy study of double-bundle ACL reconstruction using hamstring autografts, found no statistical difference in cellularity, metaplasia or cellular metabolic status between the midterm group assessed 13 to 30 months after ACLR and the long-term group assessed 31 to 62 months after ACLR. No data were presented for the first year after ACLR.

Vascularity

Hypervascularity characterized the early healing stage (between 6 and 8 weeks), during which the graft is covered with thick synovial tissue containing abundant capillary blood vessels.⁹ Rougraff et al.⁷ found a trend of

increased neovascularity in the BPTB graft biopsies during the first 2 years after ACLR, toward a gradual decrease in the later biopsies up to 78 months after reconstruction. Janssen et al.⁸ also reported an increase in vessel density in biopsy groups, but it never reached the level of normal ACL at any time during the 117 months of follow-up. In contrast, Falconiero et al.⁶ reported that biopsied portions of HT and BPTB grafts 12 months after ACLR closely resembled the normal ACL, indicating vascular maturity.

Extracellular matrix

From the early stage of the postoperative period at 6-8 weeks⁹ up to 12 months after ACLR,^{7,8} biopsy studies showed an irregular collagen orientation. After this first period, it changed into a more regular orientation, adapting to but not fully restoring the appearance of a normal ACL.^{6,8}

Fig 2. Ligamentization stages in human hamstring (*) and patellar (†) tendon grafts, as published by Pauzenberger et al.⁵ ACL, anterior cruciate ligament.

Tendinous tissue had a considerably different collagen-reducible crosslinks elution profile compared to ligamentous tissue. The cruciate ligaments showed the highest ratio between dihydroxylysinoxorleucine (DHLNL) and hydroxylysinoxorleucine (HLNL) when compared to patellar tendons or semitendinosus and gracilis tendons.³⁴ Marumo et al.³⁴ found an increase in this DHLNL/HLNL ratio over time and patterns of reducible crosslinks in BPTB and HT autografts resembling the normal ACL approximately 1 year after surgery.

Cho et al.³¹ compared electron microscopy collagen findings in double-bundle and single-bundle hamstring tendon autografts. The diameter of collagen fibrils in the reconstructed ACL with hamstring tendons was significantly smaller than that in the original hamstring tendons. Moreover, collagen fibril diameter was significantly larger in the double-bundle group, possibly leading to increased tensile strength.^{9,31}

Large-diameter collagen fibrils commonly decreased over time and eventually disappeared, leading to a change in collagen distribution from a bimodal to a unimodal pattern in ACLR using allografts and autografts.^{9,32,35} These ultrastructural changes occurred mainly during the first 2 years after ACLR for both hamstring and BPTB grafts. No evident further changes took place from this moment until 10 years after surgery.^{32,35} In contrast, Dong et al.³³ found a higher percentage of large-diameter collagen fibrils and a bimodal distribution, not changing significantly between the midterm group, 13-30 months after ACLR, and the long-term group, 31-62 months after ACLR.

Postoperative changes in MRI graft signal to noise quotient over time

SNQ was measured using a region-of-interest tool on a single image (mostly oblique coupes) in all cases. As seen in Tables 3 and 4, different SNQ/SNR calculation techniques were used across the included studies. Despite variation in absolute values, the included studies showed an initial increase of the SNQ/SNR in the postoperative period, peaking at approximately 6 months, followed by a gradual decrease over time toward direct postoperative values.^{13,20-24,26,36-38} None of the included studies included a correlation of graft SNQ and histologic findings of remodeling.

Two studies reported on the relation between SNQ and the graft bending angle.^{20,24} Tashiro et al.²⁴ determined the in vivo dynamic graft bending angles and SNQ at 6 and 24 months after anatomic ACLR using quadriceps tendon autografts in 24 patients. A steep graft bending angle was significantly correlated with high signal intensities of the proximal graft, indicating a possible negative effect on proximal graft healing. At 24 months, mean SNQ had decreased, and no difference was found between graft regions.²⁴

Of the included studies, 2 reported on differences in SNQ between for ACLR in combination with ACL remnant preservation.^{21,22} Gohil et al.²¹ compared minimal debridement of the intercondylar notch and the residual ACL stump with a standard technique using HS autografts for ACLR in a randomized controlled trial. The minimal debridement group had significantly higher SNQ at 2 months, whereas the standard group had higher signals at 6 months. At 1 year, there was no significant difference between the groups.²¹ Lee et al.²²

found higher SNQ in the first 2 periods until 4 months after ACLR in their patients in whom the ACL remnant was preserved. In period 3 (6-9 months) there was no statistical difference between the remnant-preserving or -sacrificing group. In period 4 (12-19 months) the SNQ of the remnant sacrificing group was significantly higher.²²

Relation of imaging parameters with clinical and functional outcome

Biercevicz et al.¹⁵ examined the relationship between a combination of graft volume/signal intensity and clinical parameters by means of multiple linear regression models for both BPTB and HT autografts. No data were presented for the first 3 years after ACLR. At 3 years of follow-up, volume combined with median graft signal intensity did not predict any item of the Knee Injury and Osteoarthritis Outcome Score (KOOS). However, at 5 years of follow-up, the combination of volume and signal intensity did predict KOOS-QoL ($R^2 = 0.049$, $P = 0.012$), KOOS-Sport ($R^2 = 0.37$, $P = 0.048$), KOOS-Pain ($R^2 = 0.46$, $P = 0.017$), and KOOS-Sym ($R^2 = 0.45$, $P = 0.021$). The combination of volume and signal intensity also predicted hop percentage at 3 years ($R^2 = 0.40$, $P = 0.008$) and 5 years ($R^2 = 0.62$, $P = 0.003$) of follow-up. A study by Hofbauer et al.³⁹ reported only a poor correlation between mean SNQ and KT-1000 findings with both Pearson and Spearman coefficients (not specified) at 6 months after ACLR using HS autografts in 62 patients. Li et al.¹³ used Spearman correlation analysis to investigate possible associations among several factors (International Knee Documentation Committee score, Lysholm score, Tegner score, and anterior tibial translation difference), and the MRI SNQ value of HT autografts and tibialis anterior allograft. For the autograft, the graft SNQ had a significant positive association with the anterior tibial translation difference at 3 months ($P = 0.01$). However, no other associations were found between the SNQ and clinical scores at 3, 6 and 12 months after ACLR. In a former study by the same authors, a positive correlation ($r = 0.392$, $P = 0.01$) was found between graft SNQ and the Tegner score.⁴⁰

Discussion

Although active remodeling after ACLR continues beyond 1 year after surgery, MRI signal/noise changes over time do not seem to reflect through biopsy determined remodeling stages, nor clinical and functional outcomes.

The systematic review by Claes et al.⁴ was the first to compare remodeling time frames in human grafts after ACLR. Pauzenberger et al.⁵ adapted this idea and added 1 new human study.⁸ Both reviews reported 3 phases of remodeling, consisting of an early healing phase, a phase of remodeling and a maturation or ligamentization phase. Furthermore, they proposed a slower progression

of remodeling in HT compared with BPTB autografts. However, interpretation of these results remains difficult due to a lack of strictly defined criteria for remodeling and to differences in study design and surgical techniques.⁵ Compared to Pauzenberger et al.,⁵ additional biopsy studies included in this review³¹⁻³⁵ were evaluated in a qualitative analysis on various histologic aspects (cellularity, vascularity and extracellular matrix of the human ACL graft). Although the majority of included cases were reported to be clinically stable after reconstruction, no correlation was made with histologic findings. However, based on the histologic appearance of active fibroblasts, increased vascularity and collagen fibril diameter changes, there seems to be a trend toward ongoing remodeling even beyond 1 year after ACLR.^{5,8,9,34,35} This finding could indicate insufficient mechanical properties of the graft in the first year after surgery despite clinically stable knee outcome scores and, thus, the need for an extended rehabilitation program in order to reduce the risk of ACL graft rerupture. To guide individual patients toward their optimal timing in return to sport, monitoring the remodeling process is desirable. However, the invasive nature of the biopsy procedure prevents safe and serial observation. Another potential limitation of human biopsies is the tendency to perform mainly superficial graft examination, which could influence the results because it might not represent the whole graft.^{4,5,34} Therefore, a need exists for less invasive techniques to monitor individual graft maturity after ACLR.

MRI-derived parameters predict ex vivo structural properties of the graft after ACLR in animal studies.¹⁷⁻¹⁹ However, in vitro biomechanical testing of the graft after reconstruction is not possible in humans. As a noninvasive imaging modality, MRI could potentially aid in the indirect assessment of graft maturity after ACLR. MRI is a widely used clinical tool to monitor the graft qualitatively after ACLR.⁴¹ Signal intensity, an MRI parameter for the function of tissue type and water content, has been used to evaluate the integrity and maturation of the graft.⁴² Signal changes over time represent the gradual reduction in water content and vascularity, as shown in animal studies. These changes possibly reflect the progression of remodeling.^{19,43} A growing number of studies report SNQ using background signal, instead of signal intensity alone, to take into account differing physiologic conditions such as tissue hydration.^{25,27} Furthermore, as a normalized quantification of signal intensity, the SNQ is able to compare values more reliably between patients and successive scans. Therefore, SNQ potentially reflects graft maturity better. A pattern of SNQ changes over time could be compared to biopsy findings and could even aid in the determination of graft maturity and, therewith, the timing of return to sport. The included studies all showed an initial increase in SNQ, peaking at

approximately 6 months, followed by a gradual decrease over time. However, various other underlying causes for change in MRI signal intensity should be considered. Graft type, graft bending angle and associated impingement, and ACL remnant preservation may affect graft healing and, therefore, graft signal intensity, as noted in the Results section.^{13,20-24,42,44} Furthermore, the signal intensity of the graft can vary depending on scanner hardware and MRI acquisition characteristics.^{45,46} For example, higher MRI field strength (Tesla) generates higher SNQ.⁴⁷ Single-image SNR calculation and artefacts due to the orientation variation in MRI signals are 2 more limitations when using the SNQ/SNR for graft maturity assessment.^{27,46,48} Together with differing surgical techniques and the inconsistent intervals of signal-intensity measurements of the included studies, direct comparison of the SNQ values among studies and the graft types used is complicated and statistically questionable. Therefore, no meta-analysis was performed in this systematic review. Furthermore, the postoperative SNQ pattern over time does not seem to reflect the histologic stages of remodeling reported by the studies included in Pauzenberger et al.⁵ To wit, hamstring autograft studies start their early healing phase at 6 months, whereas in BPTB autograft studies, 6 months would still reflect ongoing remodeling or early healing. Together with the above-mentioned limitations of graft SNQ, this impedes the finding of a concrete correlation between graft SNQ and histologic remodeling stages. No single study used both MRI and biopsy methods, so the future place of MRI SNQ in graft maturity assessment after ACLR is uncertain. More research using larger patient numbers and consistent imaging protocols at fixed follow-up times is needed to compare SNQ changes with histologic remodeling time frames as used in human biopsy studies. Also, feasibility studies of T2 relaxation time and diffusion tensor imaging offer potential possibilities in graft-maturity assessment.^{11,49,50} The results of our study are in line with a review by Van Dyck et al.,²⁷ who reported a more in-depth analysis of MRI signal intensity feasibility and confounders. In the present review, we were able to include 3 more MRI-related articles^{22,24,36} and provide an overview of various methods of graft maturity assessment and their relationship.

Although clinical and functional outcome measures are used to evaluate outcome and timing of return to sports after knee ligament reconstruction, there is a lack of objective criteria regarding these tests and their ability to predict graft maturity after ACLR. The determination of graft maturity based on histologic findings relies on multiple parameters.^{6-9,22,24,30-35} Therefore, clinical and functional outcomes are probably not able to predict graft maturity and structural properties directly on the basis of histologic findings. Because MRI

parameters predict animal graft maturity and structural properties,¹⁷⁻¹⁹ these imaging determinations could potentially serve as an indirect measure of graft maturity after ACLR. According to Li et al.,¹³ graft SNQ does not have the ability to predict the clinical or in vivo outcome measures in patients at 1-year follow-up. Graft SNQ shows an inordinate change during the first year after reconstruction, so it might be an unstable time to find a correlation between graft stability and knee function.¹³ The higher subscores of the KOOS in patients with larger graft volumes and lower signal intensity at 5-year follow-up were not found at the 3-year follow-up in the study by Biercevizc et al.¹⁵ This might be explained by the lack of variability in graft volume and signal intensity at this time point.¹⁵ Low number of patients with evident knee laxity in general may also contribute to the inability of relating MRI SNQ to knee stability scores. In addition, although different technical aspects of ACLR influence MRI SNQ, clinical outcomes and functional scores tend to show no significant difference in these studies.^{13,20,21} Furthermore, it is worth noting that knee stability and PROMs are also influenced by muscular strength and proprioception, which could be more important than the integrity of the ACLR as assessed by MRI. The studies included in this systematic review did not reveal an evident correlation between graft SNQ and knee-stability outcome scores at the short- and long-term follow-ups after ACLR. This also means that graft SNQ, as it stands, is not a reliable outcome to guide return to sport.

Limitations

The limitations of the present systematic review emerge mainly from the heterogeneity of the investigated data in both biopsy and imaging studies. As a result, only a descriptive and qualitative analysis of the included studies was possible. This also prevented direct correlation among the differing methods of graft maturity assessment using the today's available literature. A great number of the included studies showed risk of bias, mainly because of retrospective study designs and case series. Our research questions comprised multiple subtopics within the vast amount of literature on graft remodeling. This may have influenced completeness of search results. However, by keeping the initial search broad and through the support of various languages and the manual searches of the references used in the selected full-text studies, we believe bias was kept to a minimum. Furthermore, as an issue inherent to systematic reviews, publication bias might have been an issue.

Conclusions

The remodeling of the graft is an ongoing process, even 1 year after ACLR, based on human biopsy studies. MRI SNQ peaked at approximately 6 months,

followed by a gradual decrease over time. Heterogeneity of the MRI methods and technical restrictions used in the current literature limit prediction of graft maturity, clinical and functional outcome measures by means of MRI graft SNQ after ACLR.

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