Preoperative Synovial Tissue and Synovial Fluid Biomarkers as Predictors for Outcomes After Knee Arthroscopy and ACL Reconstruction

A Narrative Review

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Background: Biomarkers collected in synovial tissue and fluid have been identified as potential predictors of outcomes after arthroscopy.

Purpose: To provide a narrative review of the current literature that assesses the associations between preoperative biomarkers in the synovial fluid or synovial tissue and patient outcomes after knee arthroscopy.

Study Design: Narrative review.

Methods: We searched the PubMed database with keywords, "biomarkers AND arthroscopy," "biomarkers AND anterior cruciate ligament reconstruction," and "biomarkers AND meniscectomy." To be included, studies must have collected synovial fluid or synovial tissue from patients before or during arthroscopic knee surgery and analyzed the relationship of biomarkers to postoperative patient outcomes. Biomarkers were classified into 4 main categories: metabolism of aggrecan in cartilage, metabolism of collagen in cartilage (type II collagen), noncollagenous proteins in the knee, and other. When biomarker levels and outcomes were expressed with continuous variables, we abstracted the Pearson or Spearman correlation coefficients as the effect measure. If the biomarker values were continuous and the outcomes binary, we abstracted the mean or median biomarker values in those with favorable versus unfavorable outcomes. We calculated effect sizes as the difference between means of both groups divided by the standard deviation from the mean in the group with better outcomes.

Results: Eight studies were included in the review. Each study reported different patient outcomes. Biomarkers associated with metabolism of aggrecan, type II collagen metabolism, and noncollagenous proteins as well as inflammatory biomarkers had statistically significant associations with a range of patient outcomes after knee arthroscopy. Difference across studies in sample size and outcome measures precluded choosing a single biomarker that best predicted patient outcomes.

Conclusion: The findings suggest that biomarkers associated with metabolism of aggrecan, type II collagen metabolism, noncollagenous proteins, as well as inflammatory biomarkers may help surgeons and their patients anticipate surgical outcomes.

Keywords: biomarkers; knee arthroscopy; surgical outcomes; synovitis

Knee arthroscopy, a minimally invasive surgical procedure, allows surgeons to treat a wide range of knee conditions including injuries to the meniscus, anterior cruciate ligament (ACL), and other structures. Arthroscopy is performed frequently, with approximately 200,000 arthroscopic ACL reconstructions (ACLRs) and more than 400,000 arthroscopic partial meniscectomies (APMs) performed annually in the United States.[16,36]

Although most patients have improvements in pain and function for up to 10 years after ACLR, outcomes are variable[10] and patients with ACLR are at risk of developing posttraumatic osteoarthritis.[33,35] APM procedures typically show improvement in pain and functional outcomes within the first 12 months after surgery[14,20]; however, outcomes are variable after APM, and the literature concerning factors that affect these outcomes is inconsistent.[15,21,37] Patients who undergo APM are also at risk for progression of radiographic knee osteoarthritis (OA).[6,20,31]

Since knee arthroscopy is a common procedure, a diagnostic tool to predict poor short- and longer-term outcomes of

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these procedures on the basis of preoperative findings would advance care. Several studies have found demographic and imaging factors associated with outcome including age, sex, smoking history, preoperative health status, body mass index, and presence of chondral lesions.[5,6,12,20,23] However, these studies only explain about 20% of the variability in outcome.[5] In an effort to explain more of the variability in outcome and provide a better prediction for patients and surgeons, investigators have evaluated the effects on surgical outcome of biomarkers in synovial fluid, synovial tissue, serum, and urine. Synovial fluid biomarkers have been studied in patients with knee OA to detect early joint degradation and serve as a potential target for pharmaceutical therapies.[9,24] Serum and urine biomarkers were analyzed to determine their effectiveness for predicting knee OA progression in the Phase I Foundation for the National Institutes of Health case control study, but only N-telopeptide of type I collagen in urine predicted progression.[2] Osteoarthritis Research Society International published recommendations for soluble biomarker assessments in knee OA clinical trials in 2015, encouraging investigators to determine if specific biomarkers are useful for detecting earlier stages of knee OA.[17]

In addition to predicting degenerative changes of the knee, biomarkers present a potential opportunity for predicting a patient's pain and function after knee arthroscopy. We are not aware of published summaries of the role of synovial fluid and tissue biomarkers as predictors for knee arthroscopy outcomes. We aimed to provide a narrative review that identifies synovial fluid and synovial tissue biomarkers collected at baseline that can predict outcomes after multiple types of arthroscopic knee procedures. The research question guiding this review was: what is the current evidence surrounding the association of synovial fluid and synovial tissue biomarkers obtained before arthroscopic knee surgery and the outcomes of surgery, including pain, function, structure, or need for additional surgery?

METHODS

Study Selection

To address our research question, we reviewed the PubMed database for studies between 1990 and 2021, with 3 separate searches in the following order: "biomarkers AND arthroscopy," "biomarkers AND anterior cruciate ligament reconstruction," and "biomarkers AND meniscectomy." To be eligible, a study must have included patients who underwent arthroscopic knee surgery with collection of synovial fluid or synovial tissue before or during the procedure. The study needed to analyze the relationship of biomarkers obtained before or during knee arthroscopy to postoperative patient outcomes, including pain, function, cartilage damage, and the need for further surgeries. The inclusion and exclusion criteria are summarized in Table 1. We also reviewed the reference lists of all included studies for additional studies that may not have been in the original searches.

TABLE 1
Study Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
• Knee arthroscopy procedure • Synovial fluid or synovial tissue biomarkers obtained before or during surgery • Patient outcomes (pain, function, cartilage damage, and need for more surgeries) analyzed in relation to synovial fluid or tissue biomarker levels	• Joints other than the knee studied • Rheumatoid, septic, or psoriatic arthritis • Relationship between biomarkers and patient outcomes not analyzed • No arthroscopy performed • Biomarkers collected after surgery instead of before or during surgery • Animal studies • Nonclinical studies • Biomarkers studied were not obtained from synovial fluid or tissue

Biomarkers Assessed

We organized the biomarkers in knee OA according to the 4 categories used by Lotz et al[22]: biomarkers associated with the metabolism of aggrecan in cartilage, those associated with the metabolism of collagen in cartilage (type II collagen), those related to noncollagenous proteins that play a role in metabolic pathways in the knee joint, and those associated with other processes, specifically inflammation. We included all biomarkers reported in each study in this review.

Statistical Analysis

The specification of biomarker values and outcomes varied across studies. If both were expressed with continuous

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variables, we abstracted the Pearson or Spearman correlation coefficients as the effect measure. If the biomarker values were continuous and the outcomes binary, we abstracted the mean or median biomarker values in those with favorable and unfavorable outcomes. We defined the effect size as the difference between the means of both groups divided by the standard deviation of the group with better outcomes. In studies that reported Cohen *d* effect sizes, we still calculated a separate effect size to compare the same effect measure across all studies. In studies that reported median (rather than mean) values of the biomarker, we were unable to calculate a comparable effect size and simply present the median values (with interquartile ranges where available) in the comparison groups. Excel (Microsoft Corporation) was used for statistical analysis.

RESULTS

Search Results

The initial search ("biomarkers AND arthroscopy") yielded 211 results, which were narrowed down to 34 after abstract screening. After full-text review, 5 studies were found to meet eligibility criteria. We completed the same protocols for the second ("biomarkers AND anterior cruciate ligament reconstruction") and third searches ("biomarkers AND meniscectomy"). The second search yielded an additional 101 studies, and 3 studies met the eligibility criteria. However, we excluded 1 study[1] from further analysis because we were unable to abstract correlations derived from the entire cohort. The third search yielded 39 studies, none of which met eligibility criteria. One additional study was included in our analysis after a review of reference listings. Figure 1 shows the winnowing of the 351 initial studies to the 8 final studies. [7,8,11,18,25,26,29,32]

Outcomes and Biomarkers Assessed

Outcomes and biomarkers assessed for the 8 included studies are summarized in Table 2. Biomarker source was synovial fluid for all studies except for Sobue et al,[32] who used synovial tissue. Of note, 2 studies[25,26] reported follow-up surgery as a method of distinguishing favorable from unfavorable outcomes. Sobue et al[32] assessed progression in cartilage damage by comparing the number of high-grade lesions (assessed arthroscopically on 6 articular surfaces with the Outerbridge scoring system[27,38] at the initial ACLR) and at 2-year follow-up (reassessed arthroscopically

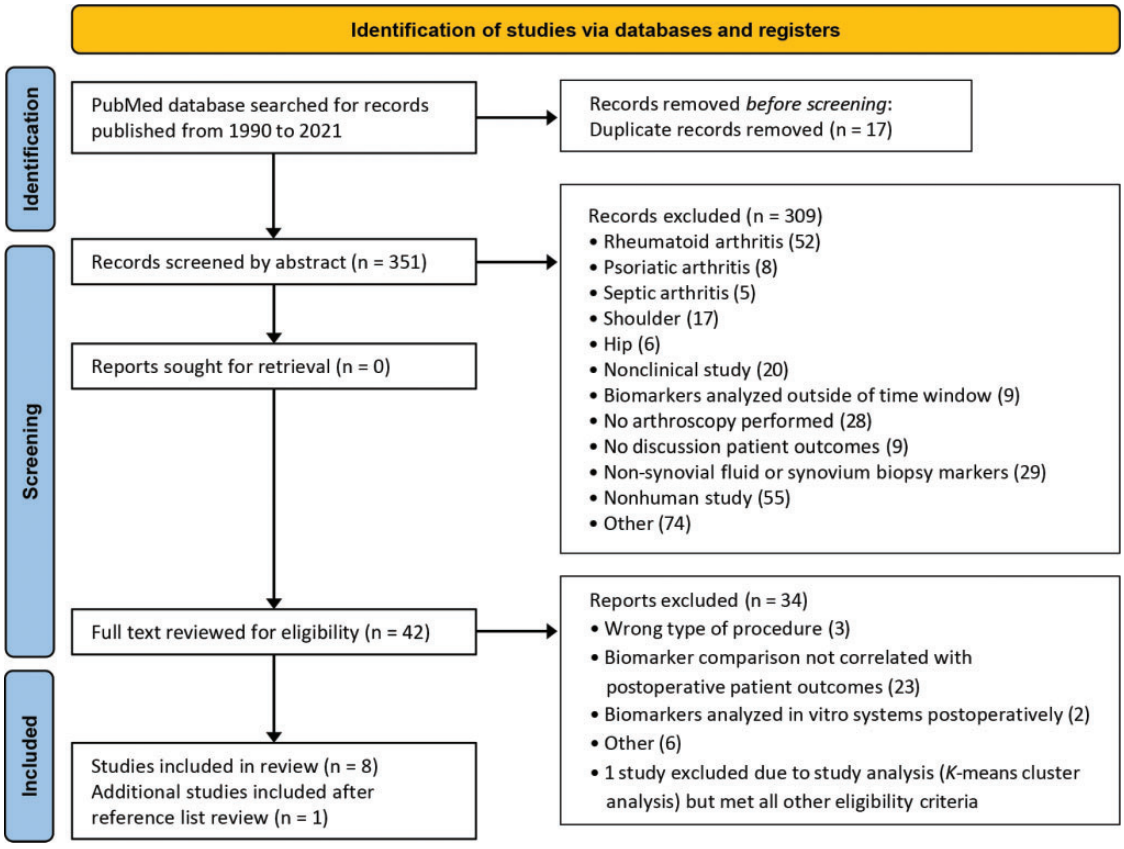

Figure 1. Flowchart of search methodology and manuscript inclusion and exclusion. The number of exclusions exceeds the number of studies excluded because some studies were excluded for multiple reasons. Three studies met two exclusion criteria after records were screened by abstract (Takahasi et al[34], No arthroscopy performed AND Other; Bresnihan et al[4], Nonclinical study AND Rheumatoid arthritis; Salvador et al[28], Rheumatoid arthritis AND Psoriatic arthritis).

TABLE 2
Summary of Patient Outcomes and All Biomarkers Tested in Each Study[a]

First Author (Year)	No. of Patients Enrolled/ Completed (Response Rate)	Outcome Assessed	Biomarkers Significantly Associated With Outcome	Biomarkers Not Significantly Associated With Outcome	Biomarker Unit of Measure[b]
Cuellar (2016)[7]	70/67 (83%)	VAS pain improvement, Lysholm, and KOOS-PS scores at 1 y after arthroscopy[c]	• VAS pain: PDGF-BB; RANTES; TIMP-3 (ACLR only) • Lysholm: PDGF-BB; RANTES; TIMP-3 (ACLR only) • KOOS-PS: MMP-3; TIMP-2	• VAS pain: MMP-3, -13; MMP3: TIMP-3; TIMP-1, -2, -3 (meniscus injuries only), and = 4; FGF-2; Eotaxin; IFNγ; IL-10; IL-1β; IL-1Ra; IL-6; MCP-1; MIP-1α and MIP-1β; TNF-α; VEGF • Lysholm: MMP-3; MMP-13; TIMP-1, -2, -3, and 4; FGF-2; Eotaxin; IFNγ; IL-10; IL-1β; IL-1Ra; IL-6; MCP-1; MIP-1α and MIP-1β; TNF-α • KOOS-PS: MMP-3; MMP-13; TIMP-1, -2, -3, and = 4; FGF-2; Eotaxin; IFNγ; IL-10; IL-1β; IL-1Ra; IL-6; MCP-1; MIP-1α and MIP-1β; TNF-α	pg/mL
Evans-Pickett (2021)[8]	38/38 (100%)	Gait biomechanics (vGRF, KFA, KEM) at 6 mo after ACLR	NR	NR	ng/mL
Gupta (2021)[11]	59/59 (100%)	VAS pain, KT-1000 arthrometer anterior knee laxity, Lysholm score, and Tegner score at 1, 2, 6, and 12 mo after ACLR	• VAS pain: IL-6, MMP-3 • Laxity: IL-6 at 1, 2, 6, and 12 mo after ACLR • Lysholm: IL-6 at 2, 6, and 12 mo after ACLR • Tegner: IL-6 at 1, 2, 6, and 12 mo after ACLR	For all outcomes: IL-1 and TNF-α	pg/mL
Lattermann (2018)[18]	22/22 (100%)	If KOOS-QOL and IKDC scores surpass PASS thresholds (62.5 and 75.9 points, respectively) 2 y after ACLR	• KOOS-QOL: IL-1α (pg/mL) • IKDC: IL-6 at 12 mo after ACLR	• KOOS-QOL: NTX-1 (nM BCE), MMP-1, COMP (μg/mL), CTX-II, sGAG (μg/mL), IL-1β (pg/mL), MMP-3, TSG-6 (U) • IKDC: NTX-1 (nM BCE), MMP-1, COMP (μg/mL), CTX-II, sGAG (μg/mL), IL-1Ra (pg/mL), IL-1β (pg/mL), MMP-3, MMP-9, TSG-6 (U)	ng/mL
Mull (2020)[25]	48/48 (100%)	Rerupture of meniscus requiring excision or fixation at 32 ± 18 mo after APM	IL-1α (pg/mL), IL-1Ra (pg/mL), MMP-9	IL-18; MMP-1; MMP-9; MMP-1; PGE2	pg/mL
Nakajima (2013)[26]	24/24 (100%)	Total or unicompartmental knee arthroplasty 2 y after arthroscopy	HGF; MMP-2; KS	C6S and C4S	nmol/mL
Scanzello (2013)[29]	28/12 (43%)	Lysholm score improvement at 16 wk and 24 mo after APM	KS (μg/mL)	IL-8, CCL21, CCL5	NR

(continued)

Table 2 (continued)

First Author (Year)	No. of Patients Enrolled/ Completed (Response Rate)	Outcome Assessed	Biomarkers Significantly Associated With Outcome	Biomarkers Not Significantly Associated With Outcome	Biomarker Unit of Measure[b]
Sobue (2017)[32]	62/62 (100%)	Arthroscopic evaluation of cartilage damage progression during tibial fixation of reconstructed ligament 2 y after ACLR	CCL19 and CCR7	C2C	nmol/mL

[a]Biomarker source was synovial fluid for all studies except for Sobue et al, who used synovial tissue. ACLR, anterior cruciate ligament reconstruction; APM, arthroscopic partial meniscectomy; C2C, collagenase-generated cleavage neoepitope of type II collagen; C4S, chondroitin-4-sulfate; C6S, chondroitin-6-sulfate; CCL, C-C chemokine ligand; CCR, C-C chemokine receptor; COMP, cartilage oligomeric matrix protein; CTX-II, C-terminal crosslinked telopeptide of type II collagen; FGF-2, fibroblast growth factor 2; HGF, hepatocyte growth factor; IFNγ, interferon γ; IKDC, International Knee Documentation Committee; IL, interleukin; IL-1Ra, interleukin 1 receptor antagonist; KEM, internal knee extension; KFA, knee flexion angle; KOOS-PS, Knee injury and Osteoarthritis Outcomes Score–Physical Function; KOOS-QOL, Knee injury and Osteoarthritis Outcomes Score–Quality of Life; KS, keratan sulfate; MCP-1, monocyte chemotactic protein 1; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; NR, not reported; NTX-1, N-terminal crosslinking telopeptide of type I collagen; PASS, Patient Acceptable Symptom State; PDGF-BB, platelet-derived growth factor BB; RANTES, regulated upon activation, normal T cell expressed and secreted; sGAG, sulfated glycosaminoglycan; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor α; TSG-6, tumor necrosis factor-stimulated gene 6 protein; U, units/mL; VAS, visual analog scale; VEGF, vascular endothelial growth factor; vGRF, vertical ground-reaction force.

[b]When biomarker units of measurement differed from other measurements in the same study, the measurement was provided in parentheses.

[c]Cuellar et al[7] included 10 patients who underwent ACL reconstruction, 35 patients who underwent arthroscopic partial meniscectomy, 20 patients who underwent arthroscopy for a combination of injured ACL and meniscus, and 5 patients who underwent knee arthroscopy for cartilage lesions.

during routine surgery for tibial staple removal). Patients in the progression group had an increased number of high-grade (grades 3 and 4) cartilage lesions during the second arthroscopy; patients without an increase in high-grade cartilage lesions in the second arthroscopy were in the non-progression group.[32] Tables 3 to 5 present the data for all biomarkers that had a statistically significant association with improvement in patient outcomes within the corresponding categories of biomarkers.

Reporting Associations between Biomarkers and Outcomes

Three studies[7,11,29] reported correlations between preoperative synovial biomarkers and postoperative patient outcomes. All correlation values are presented in Table 3. Three studies[18,25,26] compared the mean preoperative levels of synovial fluid biomarkers between patients with favorable postoperative patient outcomes and with unfavorable outcomes. Since the studies used a variety of effect measures (eg, correlation, difference in means, difference in medians), we indicate those with statistically significant associations with outcome. Two studies reported median biomarker levels associated with postoperative outcomes.[25,32]

Associations Between Biomarkers and Outcomes in APM

Higher preoperative levels of C-C chemokine receptor 7 (CCR7) and C-C chemokine ligand 19 (CCL19), both inflammatory markers, had strong correlations with greater changes in Lysholm score (score improvement) at 16 weeks (CCR7, 0.70; CCL19, 0.71) and 24 months (CCR7, 0.79; CCL19, 0.85) after arthroscopy.[29] Mull et al[25] classified patients who underwent a second knee surgery as having unfavorable postoperative outcomes and those who did not have a second surgery as having favorable outcomes. Mull et al[25] showed that those with favorable outcomes had lower preoperative levels of hepatocyte growth factor (HGF)[25] (type II collagen metabolism biomarker) and matrix metalloproteinase-2 (MMP-2)[25] (noncollagenous protein biomarker), with effect sizes of 1.3 and 0.81, respectively (Table 4). In addition to reporting mean values, Mull et al[25] also reported that the median HGF in the surgical failure group was 2.4 times higher at baseline than in the surgical success group ($P = .006$), and the median MMP-2 was 1.8 times higher in the surgical failure group ($P = .017$).

Associations Between Biomarkers and Outcomes in ACLR

Higher baseline levels of interleukin (IL)-6 (inflammatory marker) had moderate correlations with higher visual analog scale (VAS) pain scores, indicating worse pain, at all follow-up timepoints ($r = 0.364$-0.536) (Table 3).[11] Higher IL-6 levels were also associated with greater knee laxity 2, 6, and 12 months post-ACLR ($r = 0.513$, 0.626, and 0.740, respectively), with lower functional Lysholm scores (worse function) at 2, 6, and 12 months post-ACLR ($r = -0.317$, -0.714, and -0.436, respectively) and with lower

TABLE 3
Correlation Between Biomarkers and Patient Outcomes[a]

Biomarker	First Author (Year)	N	Biomarker Level (Outcome Assessed)	r^b
			Inflammatory Biomarkers	
CCR7 CCL19	Scanzello (2013)[29]	12	High levels associated with greater improvement (Lysholm score improvement at 16 wk and 24 mo after APM)	CCR7: • 16 wk: 0.704 ($P = .016$) • 24 mo: 0.790 ($P = .002$) CCL19: • 16 wk: 0.708 ($P = .05$) • 24 mo: 0.850 ($P = .004$)
IL-6	Gupta (2021)[11]	59	• High levels indicate worse outcomes (VAS pain at 1, 2, 6, and 12 mo after ACLR) • High levels indicate worse laxity (KT-1000 arthrometer anterior knee laxity at 2, 6, and 12 mo after ACLR) • High levels indicate worse outcomes (Lysholm score at 2, 6, and 12 mo after ACLR) • High levels indicate worse outcomes (Tegner score at 12 mo after ACLR)	VAS pain: • 1 mo: 0.477 ($P < .001$) • 2 mo: 0.536 ($P < .001$) • 6 mo: 0.534 ($P < .001$) • 12 mo: 0.364 ($P = .005$) Knee laxity: • 2 mo: 0.513 ($P < .001$) • 6 mo: 0.626 ($P < .001$) • 12 mo: 0.740 ($P < .001$) Lysholm score: • 2 mo: −0.317 ($P = .015$) • 6 mo: −0.714 ($P < .001$) • 12 mo: −0.436 ($P = .001$) Tegner score: • −0.570 ($P < .001$)
PDGF-BB RANTES	Cuellar (2016)[7]	70[c]	Low levels predict better outcomes (VAS pain improvement at 1 y after arthroscopy)	PDGF-BB: • −0.29 ($P < .001$) RANTES: • −0.28 ($P = .05$)
			Noncollagenous Protein Biomarkers	
MMP-3: TIMP-2 TIMP-3[d]	Cuellar (2016)[7]	70[c]	• TIMP-2: Greater ratio, worse Lysholm score (Lysholm score at 1 y after arthroscopy) • TIMP-3: Low levels predict better outcomes (VAS pain improvement at 1 y after arthroscopy)	TIMP-2: • −0.21 ($P = .05$) TIMP-3[d]: • 0.43 ($P = .05$)

[a]ACLR, anterior cruciate ligament reconstruction; APM, arthroscopic partial meniscectomy; CCL, C-C chemokine ligand; CCR, C-C chemokine receptor; IL, interleukin; MMP, matrix metalloproteinase; PDGF-BB, platelet-derived growth factor BB; RANTES, regulated upon activation normal T cell expressed and secreted; TIMP, tissue inhibitor of metalloproteinase; VAS, visual analog scale.

[b]Spearman correlation coefficients were used in Scanzello et al[29] and Gupta et al,[11] Pearson correlation coefficients were used in Cuellar et al.[8]

[c]Cuellar et al[7] included 10 patients who underwent ACLR, 35 patients who underwent arthroscopic partial meniscectomy, 20 patients who underwent arthroscopy for a combination of injured ACL and meniscus, and 5 patients who underwent knee arthroscopy for cartilage lesions.

[d]TIMP-3 was only significant in patients who underwent ACLR, but not in patients with meniscal injuries only.

Tegner activity levels (less activity) at 12 months post-ACLR ($r = -0.570$).[11] Lattermann et al[18] found that higher baseline levels of IL-1α (inflammatory biomarker), IL-1 receptor antagonist (IL-1ra; inflammatory biomarker), and MMP-9 (noncollagenous protein biomarker) were all statistically significantly associated with Knee injury and Osteoarthritis Outcomes Score–Quality of Life subscale below the Patient Acceptable Symptom State (PASS) threshold (worse outcome) with effect sizes of 3.30 ($P = .004$), 0.09 ($P = .03$), and 2.33 ($P = .01$), respectively. Higher baseline levels of IL-1α were also statistically significantly associated with International Knee Documentation Committee scores that fell below the PASS threshold (worse outcome) with an effect size of 0.38 ($P = .02$).[18] Sobue et al[32] reported that median preoperative levels of keratan sulfate (KS), chondroitin-6-sulfate (C6S), and the C6S/

TABLE 4
Mean Values Reported for Biomarkers Associated With Improved Patient Outcomes[a]

Biomarker	First Author (Year)	Surgery Type	N	Follow-up, mo	Biomarker Level and Outcome	Mean (±SD of Distribution)	Difference Between Means	Effect Size[b]
				Type II Collagen Metabolism				
HGF	Mull (2020)[25]	APM	48	32 ± 18	Higher levels of biomarker indicate worse outcome	Surgical success: 582 (±505) Surgical failure: 1236 (±978)	654	1.3
				Inflammatory				
IL-1α	Lattermann (2018)[18]	ACLR	22	24	Higher levels of biomarker indicate worse outcome (<PASS)	KOOS-QOL < PASS: 9.47 (±7.65) KOOS-QOL ≥ PASS: 2.21 (±2.20) IKDC < PASS: 5.48 (±4.56) IKDC ≥ PASS: 3.29 (±5.80)	KOOS-QOL: 7.26 IKDC: 2.19	KOOS-QOL: 3.30 IKDC: 0.38
IL-1Ra	Lattermann (2018)[18]	ACLR	22	24	Higher levels of biomarker indicate worse outcome (<PASS)	KOOS-QOL < PASS: 2593.2 (±3576.4) KOOS-QOL ≥ PASS: 2086.3 (±5507.0)	506.9	0.09
				Aggrecan Metabolism				
KS	Nakajima (2013)[26]	Multiple knee arthroscopies[c]	24	24	Larger rate of change in biomarker between baseline and 12 wk after surgery indicate worse outcomes	Favorable: 0.79 (±0.27) Nonfavorable: 0.43 (±0.20)	0.36	1.33
				Noncollagenous Proteins				
MMP-2	Mull (2020)[25]	APM	48	32 ± 18	Higher levels of biomarker indicate worse outcome	Surgical success: 188 (±89) Surgical failure: 260 (±77)	72	0.81
MMP-9	Lattermann (2018)[18]	ACLR	22	24	Higher levels of biomarker indicate worse outcome (<PASS)	KOOS-QOL < PASS: 30.99 (±35.96) KOOS-QOL ≥ PASS: 6.94 (±10.30)	24.05	2.33

[a]ACLR, anterior cruciate ligament reconstruction; APM, arthroscopic partial meniscectomy; HGF, hepatocyte growth factor; IKDC, International Knee Documentation Committee; IL-α, interleukin-1 α; IL-1Ra, interleukin-1 receptor antagonist; KS, keratan sulfate; KOOS-QOL, Knee injury and Osteoarthritis Outcomes Score–Quality of Life; MMP, matrix metalloproteinase; PASS, Patient Acceptable Symptom State (KOOS-QOL threshold, 62.5 points; IKDC threshold, 75.9 points).

[b]Effect sizes were calculated by dividing the difference between means of both groups by the standard deviation from the mean in the group with better outcomes.

[c]Nakajima et al[26] included patients who underwent synovectomy, debridement, and excision of degenerative meniscal tears, fragments of articular cartilage, or chondral flaps and osteophytes

chondroitin-4-sulfate (C4S) ratio were higher in subjects with a smaller number of high-grade cartilage lesions between baseline and implant removal at follow-up (Table 5).[32] Evans-Pickett et al[8] analyzed the association between high and low baseline concentrations of IL-6 (inflammatory biomarker) and MMP-3 (noncollagenous biomarker) and gait biomechanics and found that high synovial fluid concentrations of these markers were associated with aberrant gait biomechanics at 6 months after ACLR.

TABLE 5
Median Values Reported for Biomarkers Associated With Improved Patient Outcomes[a]

Biomarker	First Author (Year)	Surgery Type	N	Follow-up, mo	Biomarker Level and Outcome	Median Value (IQR if Reported)	P^b
Aggrecan Metabolism Biomarkers							
C6S C6S/C4S ratio KS	Sobue (2017)[32]	ACLR	62	24	Lower biomarker levels indicate worse outcomes	C6S: • Progression: 53.4 (50.9-69.4) • Nonprogression: 73.5 (60.7-99.9) C6S/C4S ratio: • Progression: 3.9 (3.2-4.4) • Nonprogression: 4.4 (3.7-5.2) KS: • Progression: 9.9 (8.3-11.4) • Nonprogression: 11.9 (10.0-16.0)	• C6S: .004 • C6S/C4S: .028 • KS: .021
Type II Collagen Metabolism Biomarkers							
HGF	Mull (2020)[25]	APM	48	32 ± 18	Higher levels indicate worse outcomes	Surgical success: 414.3 Surgical failure: 1008.0	.006
Noncollagenous Protein Biomarkers							
MMP-2	Mull (2020)[25]	APM	48	32 ± 18	Higher levels indicate worse outcomes	Surgical success: 142 Surgical failure: 261	.017

[a]ACLR, anterior cruciate ligament reconstruction; APM, arthroscopic partial meniscectomy; C4S, chondroitin-4-sulfate; C6S, chondroitin-6-sulfate; HGF, hepatocyte growth factor; IQR, interquartile range; KS, keratan sulfate; MMP, matrix metalloproteinase.
[b]*P* values reported by Sobue et al[32] and Mull et al[25] were derived from the nonparametric Mann-Whitney *U* test.

Associations Between Biomarkers and Outcomes in Multiple Knee Arthroscopies

Two inflammatory biomarkers had weak to moderate correlations with VAS scores 1 year after surgery, with lower biomarker levels associated with less pain: platelet-derived growth factor BB (PDGF-BB; $r = -0.29$) and regulated upon activation, normal T cell expressed and secreted (RANTES; $r = -0.28$).[7] Similar to Mull and colleagues,[25] Nakajima et al[26] classified patients who underwent a second knee surgery as having unfavorable postoperative outcomes and those who did not have a second surgery as having favorable outcomes. Nakajima et al[26] showed that the association between the change in KS levels (an aggrecan metabolism biomarker) between baseline and 12 weeks after surgery and improved patient outcomes (patients that did not require total knee replacement [TKR] 2 years after the initial surgery) had an effect size of 1.33.

DISCUSSION

Our principal finding in this review was that in patients undergoing APM, inflammatory (CCR7 and CCL19),[29] type II collagen metabolism (HGF),[25] and noncollagenous protein biomarkers (MMP-2)[25] were significantly associated with patient outcomes after surgery. In patients undergoing ACLR, inflammatory (IL-6, IL-1α, and IL-1ra),[11,18] noncollagenous protein (MMP-9 and tissue inhibitor of metalloproteinase [TIMP]-3),[7,18] metabolism of aggrecan biomarkers (C6S, C6S/C4S, and KS)[32] were all significantly associated with patient outcomes after surgery. In patients undergoing multiple types of knee arthroscopies, inflammatory (PDGF-BB and RANTES)[7] and noncollagenous protein biomarkers (MMP-3: TIMP-2)[7] were associated with patient outcomes after knee arthroscopy.

At baseline, higher levels of biomarkers associated with cartilage metabolism breakdown and inflammation (CCR7 and CCL19)[29] and lower expression levels of markers associated with noncollagenous proteins, type II collagen metabolism, and inflammation (PDGF-BB and RANTES)[7] were associated with improved patient outcomes. These results offer useful information for clinicians identifying which biomarkers could potentially predict patient outcomes after arthroscopy.

Knee arthroscopy patients are at a higher risk for development and progression of knee OA after surgery.[6,20,31,33,35] Boffa et al reported cluster of differentiation (CD)14, CD163, C-terminal telopeptide (CTX)I, CTXII, IL-6, procollagen type

II carboxy-terminal propeptide (PIICP) tumor necrosis factor (TNF)-α, and tumor necrosis factor-stimulated gene 6 protein (TSG)-6 as promising biomarkers associated with prognosis of knee OA (according to the BIPED classification) in patients that did not undergo arthroscopy in their systematic review including 201 synovial fluid biomarkers.[3] CD14 and CD163 were both positively associated with osteophyte progression and CTXI and CTXII were associated with rapid clinical progression.[3] Increased levels of IL-6 and TNF-α after APM and TSG-6 and PIICP in nonsurgical OA patients were associated with radiographic progression.[3] Our analysis also focused on prognostic biomarkers for predicting functional outcomes after knee arthroscopy. Although we evaluated all these prognostic biomarkers suggested by Boffa et al,[3] we identified only IL-6[8,11] and MMP-3[7,8] as significantly associated with postoperative outcomes after knee arthroscopy.[3] This may be because patients undergoing knee arthroscopy are relatively earlier in their disease process than the OA patients captured by the review by Boffa et al.[3] Although the patients in the review by Boffa et al[3] did not undergo surgery, these prognostic biomarkers reviewed might provide helpful insight for predicting OA progression after arthroscopy.

Two conflicting findings were presented in this review that were most likely due to small sample sizes in the following studies (Table 2). Although Sobue et al[32] reported that lower baseline C6S biomarker levels were significantly associated with more cartilage damage progression on arthroscopic diagnosis; Nakajima et al[26] reported that baseline C6S levels were not significantly associated with the patient's likelihood of receiving a TKR 2 years after initial knee arthroscopy. Chondroitin sulfate is a major component of the extracellular matrix of many connective tissues including cartilage and is responsible for resistance and elasticity in cartilage.[13] Since knee OA is characterized by the breakdown of cartilage, as well as structural and metabolic changes to the joint tissues including subchondral bone and synovial tissue, we hypothesize that higher levels of C6S might reflect a less-advanced knee OA joint. The results of Sobue et al reflect this hypothesis because patients with less initial C6S had worse cartilage damage after arthroscopy.[32] The lack of a statistically significant correlation between C6S levels and outcomes in Nakajima et al[26] reflect the study's small sample size and the inclusion in their cohort of patients that underwent multiple types of knee arthroscopic surgery. In contrast to Nakajima et al,[26] patients in Sobue et al[32] only underwent ACLR and the association between C6S and outcomes might be specific to ACLR. Further, the outcomes assessed in both these studies are not the same, which could also account for the different association. Sobue et al[32] assessed cartilage damage and Nakajima et al[26] assessed the need for TKR after arthroscopy. Both studies did report that KS was significantly associated with their respective patient outcomes measured.[26,32] In addition, Scanzello et al[29] reported higher baseline expression levels of inflammatory biomarkers (CCR7 and CCL19) were associated with improved patient outcomes, while Cuellar et al[7] reported lower baseline expression levels of inflammatory biomarkers (PDGF-BB and RANTES) were associated with improved patient outcomes.

Limitations

We acknowledge limitations to this review. Sample sizes of the studies we reviewed were small and outcomes and follow-up intervals varied. Further, each study reviewed different types of knee arthroscopic procedures. In addition, we recognize that the reconstructive setting during ACLR and degenerative setting during APM are different environments and might affect the types of biomarkers found in the synovial tissue or fluid. Our data render it difficult to extract a biomarker category or even specific marker found in the synovial fluid or tissue that is only present in either the reconstructive or degenerative setting.

However, to the best of the authors' knowledge, this is the first summary that relates preoperative levels of synovial tissue and fluid biomarkers with postoperative outcomes after knee arthroscopy. By applying the biomarker classification categories from Lotz et al,[22] we were able to categorize potential biomarkers that are involved with knee arthroscopy postoperative outcomes, even though a single biomarker did not show significance across all studies.

CONCLUSION

The findings of this review suggest that biomarkers associated with metabolism of aggrecan, type II collagen metabolism, noncollagenous proteins, as well as inflammatory biomarkers may help surgeons and their patients anticipate surgical outcomes. Further research is needed to improve the understanding of synovial fluid biomarkers for predicting patient outcomes after surgery, specifically how these biomarkers are associated with cartilage anabolism, metabolism, and inflammatory pathways in the knee joint. In addition, future research should focus on the difference in types of biomarkers present in the reconstructive and degenerative arthroscopic setting. This knowledge will allow clinicians to investigate potential treatments targeting these molecular processes to improve postoperative outcomes. Since there are few studies analyzing preoperative synovial fluid and tissue biomarkers for postoperative outcomes after arthroscopy, conclusions from individual studies are provisional. A high-quality study that analyzes a larger cohort of patients undergoing knee arthroscopy for synovial cartilage and inflammatory biomarkers would provide useful information for understanding which markers predict outcomes.

REFERENCES

1. Amano K, Huebner JL, Stabler TV, et al. Synovial fluid profile at the time of anterior cruciate ligament reconstruction and its association with cartilage matrix composition 3 years after surgery. *Am J Sports Med*. 2018;46(4):890-899. doi:10.1177/0363546517749834
2. Bensen G, Rogers A, Leifer V, et al. Value of using gabapentin for knee OA patients: a cost-effectiveness analysis. *Osteoarthritis Cartilage*. 2022;30:S261-S262.
3. Boffa A, Merli G, Andriolo L, Lattermann C, Salzmann GM, Filardo G. Synovial fluid biomarkers in knee osteoarthritis: a systematic review

and quantitative evaluation using BIPEDs criteria. *Cartilage*. 2021; 13(1)(suppl):82S-103S.
4. Bresnihan B. Are synovial biopsies of diagnostic value? *Arthritis Res Ther*. 2003;5(6):271-8. doi:10.1186/ar1003
5. Cleveland Clinic Sports Knee Group, Bessette MC, Westermann RW, et al. Predictors of pain and function before knee arthroscopy. *Orthop J Sports Med*. 2019;7(5):2325967119844265. doi:10.1177/2325967119844265
6. Collins JE, Losina E, Marx RG, et al. Early magnetic resonance imaging-based changes in patients with meniscal tear and osteoarthritis: eighteen-month data from a randomized controlled trial of arthroscopic partial meniscectomy versus physical therapy. *Arthritis Care Res (Hoboken)*. 2020;72(5):630-640. doi:10.1002/acr.23891
7. Cuellar VG, Cuellar JM, Kirsch T, Strauss EJ. Correlation of synovial fluid biomarkers with cartilage pathology and associated outcomes in knee arthroscopy. *Arthroscopy*. 2016;32(3):475-85. doi:10.1016/j.arthro.2015.08.033
8. Evans-Pickett A, Longobardi L, Spang JT, et al. Synovial fluid concentrations of matrix Metalloproteinase-3 and Interluekin-6 following anterior cruciate ligament injury associate with gait biomechanics 6 months following reconstruction. *Osteoarthritis Cartilage*. 2021;29(7): 1006-1019. doi:10.1016/j.joca.2021.03.014
9. Garnero P, Piperno M, Gineyts E, Christgau S, Delmas PD, Vignon E. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. *Ann Rheum Dis*. 2001;60(6):619-26. doi:10.1136/ard.60.6.619
10. Gee MSM, Peterson CDR, Zhou ML, Bottoni CR. Anterior cruciate ligament repair: historical perspective, indications, techniques, and outcomes. *J Am Acad Orthop Surg*. 2020;28(23):963-971. doi:10.5435/JAAOS-D-20-00077
11. Gupta R, Khatri S, Malhotra A, Bachhal V, Masih GD, Kaur J. Preoperative joint inflammation has no bearing on outcome of arthroscopic anterior cruciate ligament reconstruction at 1-year follow-up; a prospective study. *Indian J Orthop*. 2021;55(2):360-367. doi:10.1007/s43465-020-00150-2
12. Haviv B, Bronak S, Kosashvili Y, Thein R. Which patients are less likely to improve during the first year after arthroscopic partial meniscectomy? A multivariate analysis of 201 patients with prospective follow-up. *Knee Surg Sports Traumatol Arthrosc*. 2016;24(5):1427-1431. doi:10.1007/s00167-015-3590-z
13. Henrotin Y, Mathy M, Sanchez C, Lambert C. Chondroitin sulfate in the treatment of osteoarthritis: from in vitro studies to clinical recommendations. *Ther Adv Musculoskelet Dis*. 2010;2(6):335-348. doi:10.1177/1759720X10383076
14. Katz JN, Shrestha S, Losina E, et al. Five-year outcome of operative and nonoperative management of meniscal tear in persons older than forty-five years. *Arthritis Rheumatol*. 2020;72(2):273-281. doi:10.1002/art.41082
15. Katz JN, Wright J, Spindler KP, et al. Predictors and outcomes of crossover to surgery from physical therapy for meniscal tear and osteoarthritis: a randomized trial comparing physical therapy and surgery. *J Bone Joint Surg Am*. 2016;98(22):1890-1896. doi:10.2106/JBJS.15.01466
16. Kim S, Bosque J, Meehan JP, Jamali A, Marder R. Increase in outpatient knee arthroscopy in the United States: a comparison of National Surveys of Ambulatory Surgery, 1996 and 2006. *J Bone Joint Surg Am*. 2011;93(11):994-1000. doi:10.2106/JBJS.I.01618
17. Kraus VB, Blanco FJ, Englund M, et al. OARSI Clinical Trials Recommendations: soluble biomarker assessments in clinical trials in osteoarthritis. *Osteoarthritis Cartilage*. 2015;23(5):686-697. doi:10.1016/j.joca.2015.03.002
18. Lattermann C, Conley CE, Johnson DL, et al. Select biomarkers on the day of anterior cruciate ligament reconstruction predict poor patient-reported outcomes at 2-year follow-up: a pilot study. *Biomed Res Int*. 2018;2018:9387809. doi:10.1155/2018/9387809
19. Lattermann C, Jacobs CA, Proffitt Bunnell M, et al. A multicenter study of early anti-inflammatory treatment in patients with acute anterior cruciate ligament tear. *Am J Sports Med*. 2017;45(2): 325-333. doi:10.1177/0363546516666818
20. Li J, Zhu W, Gao X, Li X. Comparison of arthroscopic partial meniscectomy to physical therapy following degenerative meniscus tears: a systematic review and meta-analysis. *Biomed Res Int*. 2020;2020: 1709415. doi:10.1155/2020/1709415
21. Liebensteiner MC, Nogler M, Giesinger JM, Lechner R, Lenze F, Thaler M. Cartilage degeneration and not age influences the health-related quality of life outcome after partial meniscectomy. *Knee Surg Sports Traumatol Arthrosc*. 2015;23(1):26-31. doi:10.1007/s00167-013-2478-z
22. Lotz M, Martel-Pelletier J, Christiansen C, et al. Value of biomarkers in osteoarthritis: current status and perspectives. *Ann Rheum Dis*. 2013; 72(11):1756-1763. doi:10.1136/annrheumdis-2013-203726
23. Meredith DS, Losina E, Mahomed NN, Wright J, Katz JN. Factors predicting functional and radiographic outcomes after arthroscopic partial meniscectomy: a review of the literature. *Arthroscopy*. 2005; 21(2):211-223. doi:10.1016/j.arthro.2004.10.003
24. Mobasheri A, Bay-Jensen AC, van Spil WE, Larkin J, Levesque MC. Osteoarthritis year in review 2016: biomarkers (biochemical markers). *Osteoarthritis Cartilage*. 2017;25(2):199-208. doi:10.1016/j.joca.2016.12.016
25. Mull C, Wohlmuth P, Krause M, et al. Hepatocyte growth factor and matrix metalloprotease 2 levels in synovial fluid of the knee joint are correlated with clinical outcome of meniscal repair. *Knee*. 2020;27(4): 1143-1150. doi:10.1016/j.knee.2020.05.001
26. Nakajima A, Nakagawa K, Aoki Y, et al. Changes in synovial fluid biochemical markers following arthroscopic surgery in patients with knee osteoarthritis. *Rheumatol Int*. 2013;33(1):209-214. doi:10.1007/s00296-012-2374-8
27. Outerbridge R. The etiology of chondromalacia patellae. *J Bone Joint Surg Br*. 1961;43-B(4):752-757.
28. Salvador G, Sanmarti R, Garcia-Peiro A, Rodriguez-Cros JR, Munoz-Gomez J, Canete JD. p53 expression in rheumatoid and psoriatic arthritis synovial tissue and association with joint damage. *Ann Rheum Dis*. 2005;64(2):183-187. doi:10.1136/ard.2004.024430
29. Scanzello CR, Albert AS, DiCarlo E, et al. The influence of synovial inflammation and hyperplasia on symptomatic outcomes up to 2 years post-operatively in patients undergoing partial meniscectomy. *Osteoarthritis Cartilage*. 2013;21(9):1392-1399. doi:10.1016/j.joca.2013.05.011
30. Scanzello CR, McKeon B, Swaim BH, et al. Synovial inflammation in patients undergoing arthroscopic meniscectomy: molecular characterization and relationship to symptoms. *Arthritis Rheum*. 2011;63(2): 391-400. doi:10.1002/art.30137
31. Sihvonen R, Paavola M, Malmivaara A, et al. Arthroscopic partial meniscectomy for a degenerative meniscus tear: a 5 year follow-up of the placebo-surgery controlled FIDELITY (Finnish Degenerative Meniscus Lesion Study) trial. *Br J Sports Med*. 2020;54(22): 1332-1339. doi:10.1136/bjsports-2020-102813
32. Sobue Y, Kojima T, Kurokouchi K, et al. Prediction of progression of damage to articular cartilage 2 years after anterior cruciate ligament reconstruction: use of aggrecan and type II collagen biomarkers in a retrospective observational study. *Arthritis Res Ther*. 2017;19(1):265. doi:10.1186/s13075-017-1471-1
33. Sugiu K, Furumatsu T, Kodama Y, et al. Post-traumatic articular cartilage lesions increase at second-look arthroscopy following primary anterior cruciate ligament reconstruction. *Acta Med Okayama*. 2019; 73(3):223-228. doi:10.18926/AMO/56864
34. Takahashi M, Suzuki M, Kushida K, Hoshino H, Inoue T. The effect of aging and osteoarthritis on the mature and senescent cross-links of collagen in human meniscus. *Arthroscopy*. 1998;14(4):366-372. doi:10.1016/s0749-8063(98)70003-9
35. Wang LJ, Zeng N, Yan ZP, Li JT, Ni GX. Post-traumatic osteoarthritis following ACL injury. *Arthritis Res Ther*. 2020;22(1):57. doi:10.1186/s13075-020-02156-5
36. Yazdi H, Moradi A, Sanaie A, Ghadi A. Does the hyperextension maneuver prevent knee extension loss after arthroscopic anterior

cruciate ligament reconstruction? *J Orthop Traumatol*. 2016;17(4):327-331. doi:10.1007/s10195-016-0408-9
37. Yim JH, Seon JK, Song EK, et al. A comparative study of meniscectomy and nonoperative treatment for degenerative horizontal tears of the medial meniscus. *Am J Sports Med*. 2013;41(7):1565-1570. doi:10.1177/0363546513488518
38. Yoshida H, Kojima T, Kurokouchi K, et al. Relationship between preradiographic cartilage damage following anterior cruciate ligament injury and biomarkers of cartilage turnover in clinical practice: a cross-sectional observational study. *Osteoarthritis Cartilage*. 2013;21(6):831-838. doi:10.1016/j.joca.2013.03.009

APPENDIX

APPENDIX TABLE A1
Assessment of Bias for Included Studies[a]

First Author (Year)	Failure to Develop and Apply Appropriate Eligibility Criteria (Inclusion of Control Population)	Flawed Measurement of Both Exposure and Outcome	Failure to Adequately Control Confounding	Incomplete or Inadequately Short Follow-up
Cuellar (2016)[7]	• Clear inclusion and exclusion criteria applied to study population • Utilized a small control population (specimens obtained from 21 contralateral knees with no prior surgery or knee pain)	• Outcomes were measured identically in the surgical and contralateral knees • Patients underwent different types of knee arthroscopy procedures (different exposures)	Captured potential confounders and used in Pearson correlation coefficients (age, duration of symptoms, ICRS)	All participants contacted at same follow-up time regardless of whether they provided synovial fluid from contralateral knee or operative knee
Evans-Pickett (2021)[8]	• Injured ACL population was matched to control population without ACL injury; injured patients that tore their dominant ACL were matched to the same dominant knee in the control (uninjured) group • Clear inclusion and exclusion criteria for both groups	• Baseline synovial fluid biomarkers and KOOS outcomes were not collected in the uninjured group, but all other outcome measures were collected identically • Exposures were identical	• Organized ACL cohort into 2 sets of quartiles based on baseline synovial fluid biomarkers • Failed to capture prognostic factors in uninjured group on gait biomechanics outcomes (ie, current physical activity and athletic participation)	All participants were assessed for the same amount of time
Gupta (2021)[11]	• All patients included in the trial met the same inclusion and exclusion criteria • No control group, but 2 groups based on different ACLR surgical techniques (semitendinosus gracilis graft with preserved insertions and bone-patellar tendon-bone graft) • Participants further categorized based on time of presentation after injury (<6, 6-12, or >12 weeks)	• All pre- and postoperative assessments were measured identically in both groups • Surgical treatments varied between patients (different exposures)	Data means of 3 groups were compared using a 1-way ANOVA because age was normally distributed among the patient population	All patients were assessed for the same amount of time
Lattermann (2018)[18]	Used patient population from previous multicenter prospective randomized controlled trial[19] with clear inclusion and exclusion criteria	Outcomes and exposures were identical in all patients	Separate analyses were run to account for differences in how the KOOS-QOL and IKDC assess patient outcomes	All patients included in secondary analysis were assessed for the same amount of time

(continued)

Appendix Table A1 (continued)

First Author (Year)	Failure to Develop and Apply Appropriate Eligibility Criteria (Inclusion of Control Population)	Flawed Measurement of Both Exposure and Outcome	Failure to Adequately Control Confounding	Incomplete or Inadequately Short Follow-up
Mull (2020)[25]	Applied few exclusion criteria and included patients regardless of age	Outcomes and exposures were measured identical in all patients	Statistical analyses were adjusted for based on injury qualities, age, sex, BMI, location of lesion, age of rupture, presence or rupture of ACL and additional cartilage damage	All patients were assessed for the same amount of time
Nakajima (2013)[26]	Study enrolled patients undergoing intra-articular hyaluronan injections and fulfilled the American College of Rheumatology criteria for knee osteoarthritis, biased cohort	• Patients underwent different types of arthroscopies (different exposures) • Surveillance in outcomes same in all patients, regardless of arthroscopy type	Prognostic factors were not adjusted for in statistical analyses or sensitivity analyses	All participants were assessed for the same amount of time
Scanzello (2013)[29]	Used patient population from a previous trial[30] that included rigorous inclusion and exclusion criteria	All patients underwent the same type of procedure, and all outcomes were measured identically	Possible confounders (age, BMI, sex, and cartilage integrity) were accounted for in a linear effects model to determine if synovial inflammation or hyperplasia was associated independently with Lysholm scores over time	All participants in this secondary analysis were assessed for the same amount of time
Sobue (2017)[32]	Used patient population from previous trial[38] but the only inclusion criteria was patient was scheduled for ACLR and at least 500 μL of synovial fluid could be obtained from the patient	All patients underwent same type of procedure, and all outcomes were measured identically	Possible confounders were included in a multivariable logistic regression to confirm independent impact of variables on cartilage damage progression	All participants in this secondary analysis were assessed for the same amount of time

[a]In all studies, the authors used biomarker values from samples where they could obtain an adequate amount of synovial fluid or tissue for analysis. Thus, all studies have an inherent bias because not all patients had enough biospecimens to provide for analysis. ACL, anterior cruciate ligament; ACLR, anterior cruciate ligament reconstruction; ANOVA, analysis of variance; BMI, body mass index; ICRS, International Cartilage Repair Society grading scale; IKDC, International Knee Documentation Committee; KOOS, Knee injury and Osteoarthritis Outcomes Score; KOOS-QOL, Knee injury and Osteoarthritis Outcomes Score–Quality of Life.