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Synovial Fluid Profile at the Time of Anterior Cruciate Ligament Reconstruction and Its Association With Cartilage Matrix Composition 3 Years After Surgery

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Abstract

Background: Anterior cruciate ligament tears can lead to posttraumatic osteoarthritis. In addition to biomechanical factors, changes in biochemical profiles within the knee joint after injury and anterior cruciate ligament reconstruction (ACLR) may play a role in accelerating joint degeneration.

Hypothesis/Purpose: It was hypothesized that cartilage matrix composition after ACLR is associated with the degree of inflammatory response after initial injury. This study evaluated the association between the inflammatory response after injury—as indicated by cytokine, metalloproteinase, and cartilage degradation marker concentrations in synovial fluid—and articular cartilage degeneration, measured by T1ρ and T2 quantitative magnetic resonance imaging up to 3 years after ACLR.

Study Design: Cohort study; Level of evidence, 2.

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[††]References 8, 12, 14, 16, 20, 21, 38, 52, 54, 58.

Methods: Twenty-six subjects from a longitudinal cohort study who underwent ACLR at a mean 8.5 weeks after injury (range, 4–19 weeks) had synovial fluid aspirated at the time of surgery. Immunoassays quantified biomarkers in synovial fluid. T1ρ and T2 values of articular cartilage were calculated with magnetic resonance scans acquired prior to surgery and at 6 months and 1, 2, and 3 years after surgery. Pearson correlation coefficients were calculated among the various biomarkers. *K*-means clustering was used to group subjects with similar biomarker profiles. Generalized estimating equations were used to find the overall differences in T1ρ and T2 values throughout these first 3 years after surgery between the clusters while controlling for other factors.

Results: Significant and strong correlations were observed between several cytokines (interleukin 6 [IL-6], IL-8, IL-10, and tumor necrosis factor α) and 2 matrix metalloproteinases (MMP-1 and MMP-3) ($P < .05$). Moderate correlations were found among combinations of C-terminal crosslinked telopeptide type II collagen, N-terminal telopeptide, cartilage oligomeric matrix protein, and sulfated glycosaminoglycan ($P < .05$). Two clusters were generated, 1 of which was characterized by lower concentrations of cytokines (IL-6, IL-8, IL-10, tumor necrosis factor α) and MMP-1 and MMP-3 and higher sulfated glycosaminoglycan. This cluster was associated with significantly higher T1ρ and T2 values in the medial tibial and patellar cartilage over the first 3 years after ACLR.

Conclusion: At the time of ACLR surgery, profiles of synovial fluid inflammatory cytokines, degradative enzymes, and cartilage breakdown products show promise as predictors of abnormal cartilage tissue integrity (increased T1ρ and T2 values) throughout the first 3 years after surgery.

Clinical Relevance: The results suggest an intricate relationship between inflammation and cartilage turnover, which can in turn be influenced by timing after injury and patient factors.

Keywords

T1ρ; qMR; synovitis; inflammation; posttraumatic osteoarthritis; sGAG

Inflammation has recently been recognized as one of the factors potentially contributing to the development of osteoarthritis (OA) and specifically its subtype posttraumatic OA (PTOA).[11,12,47] While OA had been classically considered a noninflammatory disease, mounting evidence suggests higher-than-normal levels of inflammatory factors in a subset of patients with OA.[10,13,21,26] Studies reported significant associations among OA symptoms, cartilage break-down products, and radiologic findings, emphasizing the connection between these cellular signaling processes and disease manifestation.[28] Whether these markers are predictors of incident OA or are present as secondary processes (or both) is unknown. PTOA is unique in that the time of injury is usually known, and frequently there is joint inflammation associated with the injury. In particular, anterior cruciate ligament (ACL) injuries are a known risk factor of PTOA and offer the opportunity to study the development of PTOA from the moment of inception. ACL injuries often occur in young healthy subjects, with the average age of patients undergoing ACL reconstruction (ACLR) in the second decade.[41] These individuals usually have no signs of cartilage degeneration prior to their injury, yet the risk of early OA increases soon after the injury, with some even developing signs and symptoms of degeneration within the first 10 years after injury.[15,37,51] The incidence of PTOA is approximately 50% within 12 years after injury despite ACLR and

noted improvement in stability[36,37]; this suggests that underlying tissue biochemical and metabolic disturbances, in addition to biomechanical cause, play a role in the etiology of PTOA.

It is believed that increased and sustained inflammation activates the matrix metalloproteinases (MMPs) that digest collagen and proteoglycan components of the cartilage matrix, culminating in cartilage degeneration associated with OA.[56] Various studies have characterized synovial fluid from ACL-injured knees to understand disease processes and identify those at high risk of complications.[††] Pro- and anti-inflammatory cytokines—such as interleukin 1α (IL-1α), IL-1β, tumor necrosis factor α (TNFα), interferon γ (IFN-γ), and IL-1ra—with MMPs that are at times used as markers of cartilage degeneration were quantified in the synovial fluid at various times after human knee injury.[1,8,12,20,52] In addition, markers of cartilage and bone turnover were profiled.[10,14,38,54] One study made the observation that in vivo synovial fluid biomarkers, after acute injury, recapitulated the temporal in vitro release patterns of matrix epitope from cartilage explants stimulated with proinflammatory cytokines, in which there was an initial loss of proteoglycan and then a loss of collagen.[12] This suggests that the early phase of acute injury may be important in the pathogenesis of matrix loss. Modifying these factors at the time of injury, during surgery, and throughout recovery could decrease the rate of PTOA. Recent studies evaluated the effects of injecting anti-inflammatory agents into the joint of ACL-injured knees near the time of surgery to improve patient outcomes, including cartilage health.[17,25,49] Early follow-up has shown some improvement in patient-reported symptoms; however, follow-ups in these studies were short, with most of the focus on immediate postoperative pain management within weeks of surgery.[25]

Quantitative magnetic resonance (qMR), particularly the measurement of T1ρ and T2 relaxation times, has emerged as a possible means of evaluating compositional changes in cartilage matrix, allowing observations of potential degeneration prior to any morphologic changes.[3] This ability offers the opportunity to identify and follow disease progression years before a clinical OA diagnosis. T1ρ and T2 relaxation times correlate with proteoglycan and collagen content in cartilage, respectively, and are elevated in OA cartilage.[30,43,44] In particular, elevated T1ρ and T2 values were reported in knees after ACL injury and reconstruction, indicating potential early degeneration in such joints.[4,32,57]

There is currently no consensus on the long-term out-come of increased inflammation on cartilage health. While inflammation, chondrocyte death, and cartilage loss are well recognized immediately after injury, the point at which the scale tips toward chronic cartilage damage rather than remodeling is unclear. As part of an ongoing cohort study at our institution, we have recruited study subjects with acute ACL tears who underwent ACLR and had follow-up knee magnetic resonance imaging (MRI). A subset of this cohort had synovial fluid drawn at the time of surgery, and this is the focus of the present study. The

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purpose of our study was to profile the molecular composition of synovial fluid after ACL injury and to associate it with cartilage matrix composition during the first 3 years after surgery, as measured by T1ρ and T2 values. We hypothesized that synovial fluid biomarker profiles from ACL-injured knees, such as high proinflammatory cytokines and increased cartilage breakdown products, are associated with higher T1ρ and T2 values after ACLR.

METHODS

Subjects

This study focused on 26 subjects (from among the 53 recruited prior to ACLR as part of an ongoing cohort study) who had synovial fluid drawn at the time of but prior to the surgical intervention. These 26 subjects agreed to a one-time aspiration while under general anesthesia at the time of surgery (Figure 1). The study was approved by the institutional review board, and written informed consent was obtained from all subjects. Exclusion criteria were previous injury or surgery to either knee, known history of OA, history of inflammatory diseases (eg, rheumatoid arthritis), and multiligamentous injury requiring additional surgical procedures. Subjects whose meniscus injuries were determined to require a repair at the time of surgery were also excluded from the study at that time, since they would undergo a different rehabilitation protocol and weightbearing requirements.

Patient Surveys

Patients were asked to complete the Marx Activity Rating Scale at the time of their visits.[42] The Marx score obtained at baseline was used as an indicator of the subject's base-line activity level prior to injury, as this scoring method evaluates activity during the previous year.

MRI Protocol and Postprocessing

Study subjects were scanned after injury but before ACLR (baseline) and at 6 months and 1, 2, and 3 years after surgery. Both knees were scanned with a 3-T MRI scanner (GE Healthcare) with an 8-channel phased array knee coil (Invivo). For the present study, since synovial fluid was drawn from only the injured side, just images from the injured knee were analyzed. Protocols included (1) high-resolution 3-dimensional fast spin echo (CUBE; repetition time / echo time = 1500/26.69 ms, field of view = 16 cm, 384 × 384 matrix size, slice thickness = 0.5 mm, echo train length = 32) and (2) quantitative combined T1ρ/T2 (T1ρ time of spin-lock = 0/10/40/80 ms, spin-lock frequency = 500 Hz, field of view = 14 cm, 256 × 128 matrix size, slice thickness = 4 mm, T2 preparation echo time = 0/12.87/25.69/51.39 ms). Postprocessing was performed with MAT-LAB (MathWorks). Mean T1ρ and T2 times were calculated for cartilage and divided into 6 compartments with methods previously described.[31,33] For longitudinal analysis, all T1ρ and T2 echoes of all follow-up scans were nonrigidly registered onto the first T1ρ echo of the injured knee at baseline with elastix ITK library (Open Source Initiative) to ensure that the same regions of cartilage were compared within a subject every time.[24,50]

ACLR Surgery

All 26 study subjects underwent ACLR performed at a single institution by 1 of 4 sports fellowship–trained orthopaedic surgeons. One surgeon performed 19 of the total 26 operations, and the remaining 3 surgeons performed 2 or 3 operations each. Single-bundle ACLR with soft tissue grafts were performed with the anteromedial portal for femoral tunnel drilling. There were 17 hamstring autografts, 1 ham-string allograft, and 8 posterior tibialis allografts. Eight patients also underwent partial meniscectomy (6 lateral only, 1 medial only, and 1 medial and lateral). All study sub-jects underwent standard postoperative rehabilitation pro-grams at our sports medicine clinic.

Synovial Fluid Collection

All synovial fluid samples were obtained on the patient's day of surgery. The intra-articular fluid was aspirated in a sterile fashion without lavage or local anesthetic just prior to the ACLR. The specimens were immediately centrifuged at 15,000 rpm for 30 minutes, and the supernatant was aliquoted and stored at –80LC until time of analysis.

Biomarker Assays

Synovial fluid was analyzed with commercially available enzyme-linked immunosorbent assays (ELISA), high-performance liquid chromatography (bilirubin/biliverdin), or chemical assay (sulfated glycosaminoglycan [sGAG]). Table 1 lists all the assayed biomarkers, the processes they reflect, and the assay coefficients of variation. Due to volume limitations, assays were run in singlicate. Random samples were selected for duplicates, and any samples that were above the level of quantification or outside the standard curve were repeated at higher dilutions. Intra-assay coefficients of variation, as determined by standards and samples run in duplicates, were \12% for all assays. The multiplex Proinflammatory Panel 1 (Meso Scale Discovery) was used to measure concentrations of IL-1β, IL-2, IL-6, IL-8, TNFα, IFN-γ, IL-10, IL-12p70, IL-13, and IL-4. Human MMP 3-Plex Kit (Meso Scale Discovery) was used for MMP-1, MMP-3, and MMP-9. Commercially available individual assay kits were obtained for the following: IL-1ra (R&D Systems), IL-1α (Meso Scale Discovery), N-terminal telopeptide (NTX; Ostex International), C-terminal crosslinked telopeptide type II collagen (CTXII; Immunodiagnostic Systems), cartilage oligomeric matrix protein (COMP; Biovendor), sGAG (Kamiva), and procollagen II C-peptide (Ibex). An in house developed assay was used to measure bilirubin/biliverdin concentrations.

Image Grading System

Images at each time point were evaluated by 2 musculoskeletal radiologists using the modified Whole Organ Magnetic Resonance Imaging Score.[34,48]

Statistical Analysis

Statistical analyses were performed with SPSS (v 23; IBM). To address nonnormal distributions, the data were trans-formed with Box-Cox transformation,[45] and all subsequent analyses were performed on the transformed values. Xlstat (Addinsoft) was used for the Box-Cox transformations. All assay results that were less than the lower limit of detection

(LLOD) were imputed by the value 1/2 × LLOD, a method used previously.[1] Pearson correlation coefficients were used to determine the correlations among the biomarkers and patient information, such as age, sex, body mass index (BMI), days to surgery (the time between injury and synovial fluid aspiration), and use of allograft versus autograft.

K-means cluster analysis was performed to group the cases with similar biomarker characteristics, reducing the dimensionality of the biomarker data. A 2-cluster analysis was performed to preserve a larger number of patients within each cluster. For the purpose of clustering, the data were transformed to a scale with a range from 0 to 1 to address *k*-means clustering's susceptibility to differing scales. Independent sample *t* tests were used to describe the biomarker differences between the clusters as well as differences in age, BMI, and days to surgery. Chi-square tests were used to evaluate for differences in sex, presence of meniscus tears between the clusters, and the differences based on use of allograft or autograft. To find whether these clusters were associated with T1ρ and T2 values, generalized estimating equations (GEEs) were used to test differences between the clusters across all time points. Other covariates included in the multivariable model were age, sex, and BMI, which are known to be associated with T1ρ and T2 values.[19,22,23] Baseline Marx scores, indicative of the patient's activity level prior to injury, and partial meniscectomy at the time of ACLR were included as covariates, as they could also affect T1ρ and T2 values.[27,46] Graft type was further included in the model to evaluate whether the type of graft (allograft vs autograft) would affect T1ρ and T2 outcomes. The GEE was used to analyze the effects of each covariate independent of all other covariates on each compartment for T1ρ and T2 values. Significance was defined by $P < .05$, and 95% CIs were noted.

RESULTS

Patient Characteristics

There were 15 male and 11 female subjects with a mean ± SD age of 34.04 ± 9.5 years and BMI of 23.86 ± 3.1 kg/m^2. The number of days from injury to baseline MRI was 52.35 ± 24.7, and the days to surgery (from injury to synovial fluid collection) was 63.88 ± 27.1. A total of 23 subjects returned for their 6-month postsurgical follow-up visit, 21 for the 1-year follow-up, 19 for the 2-year follow-up, and 18 for the 3-year visit. Of the 8 subjects who did not appear for their 3-year follow-up, 1 suffered a graft tear, and 2 underwent meniscus repair; therefore, per study protocol, they dropped out of the study at that time. The rest were lost to follow-up (Figure 1). In a comparison of those who did and did not return for their 3-year follow-up, those who returned had significantly lower BMI (23.08 ± 2.6 vs 25.62 ± 3.5 kg/m^2); otherwise, there were no differences with respect to age, sex, days from injury to surgery, or concentrations of any biomarker.

Biomarker Correlations

The mean and ranges of the biomarker concentrations are presented in Table 1. Only biomarkers for which .65% of the samples were above the LLOD were included in the subsequent analyses. The biomarkers that were not included in either the correlation or cluster analyses were IL-12p70, IL-4, IL-1β, IL-2, and IL-13. There were moderate negative correlations between age and NTX ($R = -0.685$, $P < .001$), BMI and IL-10 ($R = -0.4$, P

= .043), IL-6 and days to surgery (R = −0.469, P = .016), and MMP-1 and days to surgery (R = −0.452, P = .020). There were no significant differences in the mean of any synovial fluid biomarker concentrations in the use of allograft versus autograft. Appendix Table A1 (available in the online version of this article) gives the Pearson correlation coefficients among the biomarkers. Some notable findings include significant positive correlations among cytokines and MMPs, with the strongest correlation seen between MMP-1 and MMP-3 (R = 0.87, P < .001), and among some of the cartilage and bone turnover products. Significant negative correlation was seen only between sGAG and MMP-3.

Biomarker Comparisons Between Clusters

K-means cluster analysis grouped the subjects into 2 clusters, within which biomarker characteristics were similar. Table 2 shows differences by clusters. The clusters did not differ significantly by age, sex, BMI, days to surgery, presence of meniscus tear, or use of allograft versus autograft. Cluster 1 showed an overall higher inflammatory profile as compared with cluster 2; the only exception was sGAG, which was lower in cluster 1. Therefore, cluster 1 was referred to as the “inflammation” cluster, while cluster 2 was referred to as “high sGAG” cluster.

Biomarker Cluster Association With qMR Over 3 Years

Appendix Table A2 shows the mean and SD for T1ρ and T2 relaxation times at each time point. GEE analysis of these T1ρ and T2 relaxation times is demonstrated in Table 3. For T1ρ, increased age was significantly associated with increased relaxation times in the medial femur, lateral femur, medial tibia, patella, and trochlea. Female sex was associated with significantly higher relaxation times in all compartments. Higher BMI (independent of sex) was associated with lower T1ρ in the medial femur, medial tibia, and the patella. Meniscectomy was associated with higher T1ρ in the medial tibia. Higher baseline Marx was also associated with higher T1ρ in the medial femur, lateral femur, medial tibia, patella, and trochlea. The use of allograft was associated with higher T1ρ in the lateral femur and the lateral tibia. The high sGAG cluster had significantly higher T1ρ in the medial tibia and the patella.

For T2, age was associated with higher relaxation times in the medial femur, lateral femur, medial tibia, and trochlea. Female sex, like T1ρ, was associated with higher values in all compartments. Higher BMI was associated with lower T2 in the medial femur and the medial tibia. A higher T2 was associated with meniscectomy in the medial tibia and trochlea. Higher Marx was associated with higher T2 in the medial femur, medial tibia, patella, and trochlea. Higher T2 was seen in the high sGAG cluster in the medial tibia and patella.

DISCUSSION

To the best of our knowledge, this is the first study reporting an association between biochemical profiles of synovial fluids after ACL injury and longitudinal cartilage matrix composition measured by T1ρ and T2 qMR. We determined the biochemical profiles of synovial fluids obtained from the knee joint at the time of surgery after ACL injury, and a *k*-

cluster analysis revealed 2 groups of patients who varied on the basis of level of inflammation and sGAG concentration. Analyses of these clusters with cartilage matrix composition—as measured by T1ρ and T2 values up to 3 years after reconstruction—demonstrated an association between higher sGAG concentrations and worse matrix composition, as indicated by higher T1ρ and T2 values, particularly in the medial tibial plateau and patella. While it is generally accepted that biomarkers are elevated after injury, our study is unique because we correlated synovial fluid biomarkers—specifically sGAG, which is released with cartilage degradation—obtained at the time of surgery with cartilage composition over the subsequent 3 years.

The concentrations of many of the biomarkers observed in this study were similar to those in published reports in the literature that analyzed synovial fluid aspirated from ACL-deficient knees within a similar time frame (within days from injury to 6 months after injury).[8,21,52] However, a few biomarkers, such as IL-1β and IL-2, for which the majority of samples fell below the LLOD in our study, were more readily detected in other studies.[20,52] This could be due to the observation that some of these marker levels are highest in the acute phase after injury, whereas our samples represented later time points, when the concentration of some of these markers would have been expected to be lower.[8–11] Likewise, IL-6 and MMP-3 were both negatively correlated with days from injury to surgery. We were surprised that we did not find more correlations with days to surgery. However, although not significant, all biomarkers except IL-1ra and CTXII showed a negative correlational trend with days to surgery. Because the synovial fluid was drawn at the time of surgery (prior to graft placement), the type of graft would not have affected the inflammatory markers.

There were strong positive correlations between the cytokines and MMPs and moderate positive correlations among sGAG, NTX, CTXII, and COMP. While the negative correlation between sGAG and MMP-3 was the only significant correlation between cytokines/MMP biomarkers and bone/cartilage turnover products, consistently negative correlation coefficients were observed for other pairs—for example, sGAG and TNF-α or COMP and IL-6. Our cluster analysis showed results consistent with these correlations, with significantly higher cytokines and MMPs but lower sGAG for the inflammation cluster and vice versa for the high sGAG cluster. The negative correlation between cytokines and sGAG was surprising, as we would hypothesize that higher inflammation would lead to more cartilage breakdown and its products. However, our data represent a snapshot taken after injury, and there may be a time discrepancy between the presence of enzymes and the increase in breakdown products. It is also possible that intense early inflammation may deplete cartilage glycosaminoglycan (GAG) and therefore exhaust sGAG loss or inhibit GAG synthesis; this possibility is supported by in vitro exposure of cartilage explants to inflammatory cytokines and by acute joint injury in animal models and humans, as characterized by an early spike in GAG loss, followed by an abrupt decline.[12,35] In addition, it is possible that with a larger study, these biomarkers could be shown to contribute additional information to the clusters. Characteristically, GAGs are lost quickly after injury; an initial release of GAGs appears to be due to mechanical damage followed by loss owing to enzymatic degradation with increased GAG release to the media for up to 4 days for higher magnitude impacts.[55] In our study, higher sGAG detected at a much later time point may represent a much higher catabolic response than was present immediately after injury.

In our GEE analysis, older age, female sex, partial meniscectomy, baseline activity, use of allograft, and higher sGAG concentrations were all associated with higher T1ρ and T2 values. Age and activity have been shown to increase T1ρ and T2 values,[19,39] but the effects of sex on cartilage health remain unclear after injury.[7,40] In our study, female sex was a strong predictor of higher T1ρ and T2 values in multiple compartments, with 2- to 4-ms increase in T1ρ and T2 relaxation times in all regions. Interestingly, the medial tibia was the only region where all factors except for the use of allograft showed significant association with T1ρ and T2, including high sGAG. A higher concentration of sGAG may indicate the knee's tendency for cartilage breakdown, in excess of repair, after ACLR. We also found it interesting that this finding between clusters was in the medial tibia, as PTOA is often observed in the medial compartment after ACL injury.[2,5]

There were several surprising observations in the GEE analysis. First, we found that BMI was associated with lower T1ρ. This was unexpected, as higher BMI is linked to increased risk of OA.[22,23] But our study had only 1 subject with BMI >30; with such a narrow range, it may be difficult to draw conclusions based on our results regarding the effects of BMI on cartilage after ACLR. In addition, since the BMI of athletes does not reflect adiposity, a higher BMI may relate to greater muscle strength and cartilage-protective mechanisms, as manifested by lower relaxation times; accordingly, the role of fat mass and muscle mass on OA is still debated.[6,53] Further analyses regarding the relationship among BMI, muscle mass, activity, and cartilage composition will need to be performed. Second, the use of allograft was associated with higher T1ρ values in the lateral compartments only, although it did approach significance in the medial femur ($P = .051$). While the use of allograft may alter the biomarker profile of the synovial fluid after surgery, our synovial fluid was drawn prior to the introduction of a graft into the joint. A future study would need an analysis of synovial fluid after surgery to elucidate the relationship among allograft use, biomarkers, and cartilage health. Third, we had initially hypothesized that inflammation is associated with worse cartilage outcomes, but the high sGAG group, which was associated with higher T1ρ and T2 values, had lower concentrations of inflammatory markers. This is in contrast to our hypothesis that higher inflammation would lead to more cartilage breakdown (ie, higher T1ρ and T2 relaxation times). However, as mentioned previously, there may be a time dependence on the levels of inflammatory markers, and it is very possible that the actual cartilage breakdown, as represented by sGAG, may be more indicative of cartilage degeneration and more predictive for long-term cartilage health rather than the inflammatory markers themselves, whose concentrations may have waned by the time of our synovial fluid harvest. The concentration of inflammatory markers alone may not reflect the entire process, as there may be individual variation in cartilage susceptibility to inflammation and metalloproteinases that lead to cartilage degradation. This may explain why the presence of sGAG—-which is a direct measure of proteoglycan fragmentation and loss and, therefore, cartilage degradation—is associated with higher relaxation times at 3 years after ACLR.

One aspect that was not accounted for in our study was the use of nonsteroidal anti-inflammatory drugs (NSAIDs) around the time of injury and surgery. As NSAIDs are readily available over the counter, our subjects may have taken them prior to initial presentation to our clinic and up to the time of surgery. NSAIDs were also routinely prescribed after surgery on an as-needed bases. A study by Gallelli et al[18] observed significantly decreased

inflammatory markers in synovial fluids of patients with OA who took systemic NSAIDs prior to their total joint operations. It is very possible that those who took NSAIDs in our study had lower inflammatory profiles. This pilot study nevertheless suggests that an early biological consequence of ACL injury is associated with structural knee joint degeneration. We are unable to make conclusions regarding causation at this point. Having tools to discern early risk for poor outcomes, we are better prepared to evaluate for proximal causes and, in the future, investigate ancillary treatment effects, such as the use of NSAIDs or steroids on joint injury–related inflammation and inflammatory markers.[25,29] The optimal timing of these and surgical interventions need to be further elucidated to improve outcomes after joint injury.

Our study is limited by the modest size of the cohort. One or 2 patients were lost to follow-up per interval, and an additional 3 patients were lost to follow-up owing to graft failure and meniscus repair. A larger study could allow subgroup analysis of individuals with graft failure or meniscus repair and shed more light into the effects of synovial fluid biomarkers. In addition, a larger study would be needed to evaluate the independent effects of inflammation, surgical technique, timing of return to sports and type of sport, and impact on the outcomes of the operated knee. The size of the cohort also made correcting for multiple testing a challenge. The large correlational matrices and testing for multiple compartments would require a larger cohort to confirm our findings. Our cohort included only skeletally mature adults. While these were young active adults with no prior diagnosis of OA, ACLR is frequently performed on individuals who are much younger. Since it is possible that skeletally immature individuals, whose physes are still open or recently closed, may have a different inflammatory response than that of adults, our current findings may be applicable only to subjects in the same age group. While the entire cohort study did include a control group, we were unable to obtain synovial fluid from this group, as synovial fluid from healthy subjects could not be obtained without inducing unnecessary pain and discomfort with the methods employed in this study (ie, direct aspiration, no local anesthetic to avoid contamination).

This study was also limited by the ability to collect synovial fluid only at the time of surgery and by the varying time frames among subjects between injury and surgery. In addition, we do not have the synovial fluid profiles from the time after surgery, which may have illuminated the issue of secondary injury attributed to the surgical procedure. In the future, it may be necessary to consider evaluating biomarkers at an earlier time point after injury to fully understand the effects of early inflammation on cartilage damage. There are other biomarkers that may be of interest in investigating PTOA, such as hyaluronic acid and lubricin. Longitudinal characterization of the fluid after ACLR may be necessary to fully understand the connection between the synovial fluid microenvironment and cartilage health. Finally, a longer follow-up would be necessary to truly link the findings to PTOA development based on a radiographic diagnosis.

In summary, knee joint inflammation was prevalent following ACL injury. Higher synovial fluid sGAG concentrations at the time of surgery were associated with worse cartilage composition during the first 3 years after surgery based on T1ρ and T2 qMR. Our results provide objective evidence that suggests how increased cartilage breakdown products at the

time of surgery, which may be accompanied by the presence of an inflammatory response, place the joint on the trajectory to PTOA. Recruiting a larger cohort, drawing synovial fluid at multiple time points, and controlling for factors such as activity and NSAID use could help elucidate the role of inflammation and cartilage breakdown in the development of OA, which may in turn open opportunities for interventions and preventive strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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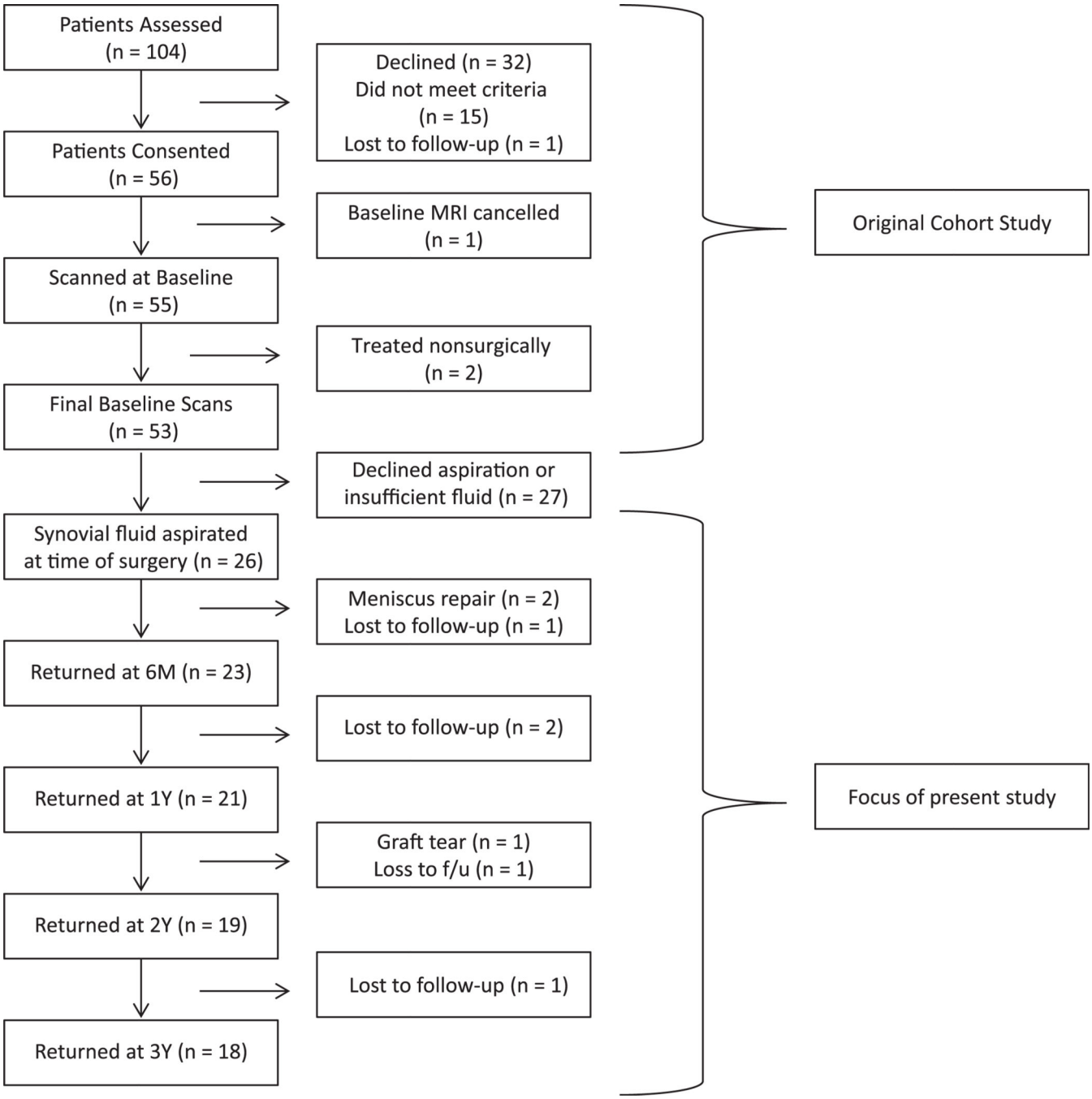

Figure 1.
Flow diagram of study subjects. f/u, follow-up.

TABLE 1

Synovial Fluid Biomarker Concentrations[a]

Process: Biomarker	Mean (Range)	CVs, %[b]	>LLOD, No. (%)
Proinflammatory, pg/mL			
IL-1α	0.61 (0.005–9.44)	8.1	21 (81)
IL-1β	0.42 (0.22–5.44)	4.6	1 (4)
IL-2	0.22 (0.1–0.94)	4.9	12 (46)
IL-6	94.11 (0.68–2162.12)	5.2	26 (100)
IL-8	22.97 (7.56–86.24)	4.5	26 (100)
TNF-α	2.11 (0.63–24.51)	6.0	26 (100)
Anti-inflammatory, pg/mL			
IL-1ra	247.52 (88.95–657.46)	4.6	26 (100)
IFN-γ	5.59 (0.45–108.75)	4.0	17 (65)
IL-10	0.66 (0.04–10.52)	4.3	24 (92)
IL-12p70	0.19(0.12–0.80)	8.1	7 (27)
IL-13	0.87 (0.80–1.80)	10.4	2 (8)
IL-4	0.24 (0.02–5.0)	11.9	8 (31)
Bone turnover: NTX, nM BCE	11.18 (4.29–21.67)	2.1	26(100)
Cartilage degeneration			
CTXII, ng/mL	0.64 (0.24–1.52)	2.9	26 (100)
COMP, mg/mL	23.46 (14.17–34.83)	2.0	26 (100)
sGAG, mg/mL	41.74 (5.37–127.5)	2.4	26 (100)
MMP-1, μg/mL	468,685.7 (39,700.2–1,308,370.9)	3.4	26 (100)
MMP-3, μg/mL	3,255,538.3 (656,372.0–10,065,822.4)	2.0	26 (100)
MMP-9, pg/mL	13,609.8 (686.95–170,649.6)	8.3	26 (100)
Collagen type II synthesis: CPII, ng/mL	799.56 (246.27–1371.2)	2.0	26 (100)
Hemarthrosis: bilirubin 1 biliverdin, μmol/L	4.26 (0.5–12.4)	3.7	19 (73)

[a]BCE, bone collagen equivalent; COMP, cartilage oligomeric matrix protein; CPII, procollagen II C-peptide; CTXII, C-terminal crosslinked telopeptide type II collagen; CV, coefficient of variation; IFN-γ, interferon; IL, interleukin; LLOD, lower limit of detection; MMP, matrix metalloproteinase; NTX, N-terminal telopeptide; sGAG, sulfated glycosaminoglycan; TNF-α, tumor necrosis factor α.

[b]CVs are intra-assay based on standards, controls, and random samples.

TABLE 2

Demographic and Biomarker Mean Values for the 2 Clusters Resulting From K-Cluster Analysis[a]

	Inflammation Cluster (n = 10)	High sGAG Cluster (n = 16)	*P* Value[b]
Age, y	35.6 ± 7.9	33.1 ± 10.5	.518
Body mass index, kg/m^2	23.4 ± 2.0	24.2 ± 3.6	.533
Days to surgery	53.5 ± 14.5	70.4 ± 31.4	.125
Male:female, No.	6:4	9:7	>.99
Allograft:autograft, No.	3:7	6:10	>.99
Subjects without meniscus tears, No.	7	6	.226
Biomarker, pg/mL[c]			
IL-1ra	258.78 ± 202.85	240.49 ± 140.12	.774
IL-1α	0.30 ± 0.32	0.79 ±2.32	.656
IFN-γ	12.75 ± 33.77	1.11 ± 0.80	.075
IL-10	1.39 ± 3.21	0.20 ± 0.10	.005[d]
IL-6	235.45 ± 677.13	5.77 ± 5.02	<.001[d]
IL-8	34.02 ± 21.07	16.06 ± 5.73	.003[d]
TNF-α	3.82 ± 7.27	1.04 ± 0.27	<.001[d]
NTX, nM BCE	10.03 ± 2.61	11.90 ± 4.30	.235
CTXII, ng/mL	0.53 ± 0.27	0.71 ± 0.38	.165
COMP, μg/mL	20.79 ± 4.30	25.13 ± 6.31	.106
sGAG, μg/mL	26.10 ± 14.4	51.51 ± 28.79	.003[d]
MMP-1	733,229.56 ± 325,378.1	303,345.81 ± 266,028.75	.001[d]
MMP-3	5,355,011.97 ± 2,703,193.1	1,943,367.32 ± 1,417,831.36	<.001[d]
MMP-9	10,049.67 ± 11,225.9	15,834.93 ± 41,490.24	.309
CPII, ng/mL	748.43 ± 242.26	831.53 ± 303.09	.543
Bilirubin 1 biliverdin, μmol/L	5.19 ± 3.71	3.69 ± 3.84	.147

[a]Values are presented as mean ± SD, unless noted otherwise. BCE, bone collagen equivalent; COMP, cartilage oligomeric matrix protein; CPII, procollagen II C-peptide; CTXII, C-terminal crosslinked telopeptide type II collagen; IFN-γ, interferon; IL, interleukin; MMP, matrix metalloproteinase; NTX, N-terminal telopeptide; sGAG, sulfated glycosaminoglycan; TNF-α, tumor necrosis factor a.

[b] Difference between clusters.

[c] Measured as pg/mL, unless noted otherwise.

[d] $P < .05$.

TABLE 3

Association of Magnetic Resonance Imaging Outcomes (T1ρ and T2) With Demographic and Cluster Characteristics Over All Time Points[a]

	T1ρ, ms				T2, ms			
	Medial Femur		Lateral Femur		Medial Femur		Lateral Femur	
Predictor	***B* (95% CI)**	***P* Value**	***B* (95% CI)**	***P* Value**	***B* (95% CI)**	***P* Value**	***B* (95% CI)**	***P* Value**
Age	**0.08 (0.02 to 0.13)**	**.011**	**0.17 (0.08 to 0.26)**	**<.001**	**0.07 (0.003 to 0.14)**	**.041**	**0.15 (0.08 to 0.22)**	**<.001**
Sex [b]	**2.90 (1.73 to 4.07)**	**<.001**	**2.77 (1.38 to 4.16)**	**<.001**	**3.10 (1.81 to 4.39)**	**<.001**	**2.32 (1.06 to 3.59)**	**<.001**
BMI	**–0.33 (–0.53 to –0.13)**	**.002**	–0.17 (–0.44 to 0.11)	.25	**–0.26 (–0.42 to –0.10)**	**.001**	–0.19 (–0.41 to 0.03)	.09
Meniscectomy [c]	0.19 (–1.60 to 1.98)	.83	–0.62 (–2.75 to 1.51)	.57	–0.58 (–1.91 to 0.75)	.39	–0.82 (–2.55 to 0.90)	.35
Marx	**0.45 (0.23 to 0.68)**	**<.001**	**0.30 (0.03 to 0.58)**	**.031**	**0.27 (0.02 to 0.53)**	**.038**	0.24 (–0.01 to 0.49)	.06
Allograft [d]	1.23 (–0.01 to 2.44)	.051	**1.22 (0.17 to 2.28)**	**.023**	0.83 (–0.28 to 1.95)	.14	0.70 (–0.39 to 1.79)	.21
Cluster [e]	1.36 (–0.06 to 2.80)	.06	0.61 (–0.95 to 2.16)	.44	0.30 (–1.02 to 1.63)	.65	0.05 (–1.24 to 1.34)	.94
	Medial Tibia		**Lateral Tibia**		**Medial Tibia**		**Lateral Tibia**	
Age	**0.10 (0.01 to 0.20)**	**.034**	0.09 (–0.08 to 0.26)	.32	**0.99 (0.01 to 0.19)**	**.027**	0.02 (–0.10 to 0.14)	.71
Sex [b]	**1.84 (0.18 to 3.51)**	**.030**	**4.04 (2.26 to 5.81)**	**<.001**	**1.79 (0.29 to 3.29)**	**.02**	**3.44 (2.14 to 4.75)**	**<.001**
BMI	**–0.63 (–0.91 to –0.36)**	**<.001**	–0.01 (–0.28 to 0.27)	.97	**–0.44 (–0.70 to –0.18)**	**.001**	–0.02 (–0.20 to 0.16)	.82
Meniscectomy [c]	**3.04 (1.37 to 4.70)**	**<.001**	0.62 (–0.99 to 2.33)	.45	**2.24 (1.01 to 3.48)**	**<.001**	0.99 (–0.12 to 2.11)	.08
Marx	**0.59 (0.25 to 0.92)**	**.001**	0.26 (–0.26 to 0.77)	.33	**0.44 (0.17 to 0.71)**	**.002**	–0.01 (–0.35 to 0.36)	.97
Allograft [d]	0.08 (–1.43 to 1.59)	.92	**1.05 (0.02 to 2.08)**	**.045**	0.62 (–0.54 to 1.79)	.30	0.31 (–0.58 to 1.19)	.50
Cluster [e]	**3.29 (1.44 to 5.14)**	**.001**	–0.11 (–1.68 to 1.46)	.89	**1.48 (0.13 to 2.83)**	**.032**	–0.28 (–1.42 to 0.85)	.62
	Patella		**Trochlea**		**Patella**		**Trochlea**	
Age	**0.20 (0.05 to 0.36)**	**.012**	**0.14 (0.04 to 0.25)**	**.007**	0.07 (–0.05 to 0.19)	.27	**0.1 (0.03 to 0.18)**	**.007**
Sex [b]	**4.17 (2.56 to 5.78)**	**<.001**	**4.50 (3.29 to 5.72)**	**<.001**	**3.96 (2.53 to 5.39)**	**<.001**	**3.69 (2.51 to 4.87)**	**<.001**
BMI	**–0.26 (–0.52 to –0.01)**	**.044**	–0.15 (–0.42 to 0.12)	.26	–0.11 (–0.31 to 0.09)	.29	–0.15 (–0.34 to 0.04)	.12
Meniscectomy [c]	0.13 (–2.15 to 2.42)	.91	–0.88 (–2.71 to 0.95)	.35	0.08 (–2.01 to 2.16)	.94	**–1.59 (–2.89 to –0.29)**	**.017**
Marx	**0.64 (0.30 to 0.99)**	**<.001**	**0.53 (0.28 to 0.79)**	**<.001**	**0.40 (0.07 to 0.73)**	**.019**	**0.38 (0.14 to 0.62)**	**.002**

	T1ρ, ms				T2, ms			
	Medial Femur		Lateral Femur		Medial Femur		Lateral Femur	
Predictor	*B* (95% CI)	*P* Value	*B* (95% CI)	*P* Value	*B* (95% CI)	*P* Value	*B* (95% CI)	*P* Value
Allograft [d]	0.98 (–0.20 to 2.17)	.10	0.72 (–0.52 to 1.95)	.26	0.94 (–0.20 to 2.08)	.11	0.58 (–0.80 to 1.95)	.41
Cluster [e]	**2.46 (0.68 to 4.24)**	**.007**	1.09 (–0.35 to 2.53)	.14	**1.74 (0.11 to 3.36)**	**.037**	0.35 (–0.88 to 1.59)	.58

[a] Each entry indicates the increase in relaxation time (ms) for every unit increase of that factor. Bold indicates $P < .05$. BMI, body mass index.

[b] A positive *B* value indicates that females have higher relaxation times.

[c] A positive *B* value indicates higher relaxation times for those who had meniscectomy.

[d] A positive *B* value indicates higher relaxation times for those with allograft versus those with autograft.

[e] A positive *B* value indicates higher relaxation times in the high sulfated glycosaminoglycan group.