

# Dysregulated Inflammatory Response Related to Cartilage Degradation after ACL Injury

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## ABSTRACT

JACOBS, C. A., E. R. HUNT, C. E.-W. CONLEY, D. L. JOHNSON, A. V. STONE, J. L. HUEBNER, V. B. KRAUS, and C. LATTERMANN. Dysregulated Inflammatory Response Related to Cartilage Degradation after ACL Injury. *Med. Sci. Sports Exerc.*, Vol. 52, No. 3, pp. 535–541, 2020. **Purpose:** Elevated synovial fluid (SF) concentrations of proinflammatory cytokines, degradative enzymes, and cartilage breakdown markers at the time of anterior cruciate ligament (ACL) reconstruction are associated with worse postoperative patient-reported outcomes and cartilage quality. However, it remains unclear if this is due to a more robust or dysregulated inflammatory response or is a function of a more severe injury. The objective of this study was to evaluate the association of the molecular composition of the SF, patient demographics, and injury characteristics to cartilage degradation after acute ACL injury. **Methods:** We performed a cluster analysis of SF concentrations of proinflammatory and anti-inflammatory cytokines, and biomarkers of cartilage degradation, bony remodeling, and hemarthrosis. We evaluated the association of biomarker clusters with patient demographics, days between injury, Visual Analogue Scale pain, SF aspirate volumes, and bone bruise volumes measured on magnetic resonance imaging. **Results:** Two clusters were identified from the 35 patients included in this analysis, dysregulated inflammation and low inflammation. The dysregulated inflammation cluster consisted of 10 patients and demonstrated significantly greater concentrations of biomarkers of cartilage degradation ( $P < 0.05$ ) as well as a lower ratio of anti-inflammatory to proinflammatory cytokines ( $P = 0.053$ ) when compared with the low inflammation cluster. Patient demographics, bone bruise volumes, SF aspirate volumes, pain, and concomitant injuries did not differ between clusters. **Conclusions:** A subset of patients exhibited dysregulation of the inflammatory response after acute ACL injury which may increase the risk of posttraumatic osteoarthritis. This response does not appear to be a function of injury severity. **Key Words:** KNEE, INFLAMMATION, CYTOKINE, CARTILAGE, BIOMARKER

After traumatic injury, increased cytokine burden and a dysregulated inflammatory response are associated with severe complications, such as multiple organ dysfunction, wound complications, and longer intensive care and hospital stays (1–3). As a consequence, some in the orthopedic trauma research community have investigated individual patient's immunologic response, damage-associated molecular patterns and injury characteristics. Identifying “Precision Injury Signatures” may not only determine if there is an association with short-term complications for polytraumatized patients (ClinicalTrials.gov Identifier: NCT0372730) but also if these injury signatures may influence diagnosis and treatment in the future (3,4).

Although life-threatening complications are extremely rare after acute anterior cruciate ligament (ACL) injury, approximately

50% of patients develop posttraumatic osteoarthritis (PTOA) within 15 yr after injury (5–7). The ACL injury initiates an innate immunologic response resulting in a cascade of cytokine and catabolic enzyme activity that is associated with increased biomarkers of cartilage turnover (8–12). Specifically, after acute ACL injury significant increases in synovial fluid concentrations of proinflammatory cytokines interleukin (IL)-1 $\alpha$  and IL-1 $\beta$ , catabolic enzymes matrix metalloproteinase-3 (MMP-3), and biomarkers of cartilage breakdown (C-terminal crosslinked telopeptide of type II collagen [CTXII], cartilage oligomeric matrix protein [COMP]) and bone turnover (C-terminal crosslinked telopeptide of type I collagen [CTXI], N-terminal crosslinked telopeptide of type I collagen [NTXI]) have been reported (9,11,13). Although inflammatory and chondrodegenerative biomarkers are elevated after ACL injury and reconstruction (10,11,14), it remains unknown why roughly half of ACL-injured patients demonstrate an early onset of PTOA whereas the other half do not.

Elevated proinflammatory cytokine, degradative enzyme, and cartilage breakdown marker concentrations in the synovial fluid at the time of ACL reconstruction have been associated both with worse patient-reported outcomes and cartilage quality 2 to 3 yr after surgery (15,16). Increased synovial fluid concentrations of IL-1 $\alpha$  and MMP-9 at the time of ACL reconstruction have been associated with worse patient reported outcomes at a

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minimum of 2 yr after surgery (16). In addition, increased synovial fluid concentrations of a sulfated glycosaminoglycan (sGAG), a biomarker of cartilage degradation, at the time of surgery have been associated with inferior cartilage quality (i.e., increased T1 and T2 wavelengths on magnetic resonance imaging [MRI]) 3 yr after surgery (15). However, it remains unclear if elevated cytokine expression and increased synovial fluid concentrations of cartilage breakdown markers on the day of surgery in a subset of patients are related to a greater, more persistent inflammatory response or whether these concentrations are the result of a more severe initial injury. For example, results from a recent animal study suggest that the magnitude and duration of bone marrow edema after ACL injury may be related to the forces borne by the joint at the time of injury (17). These knowledge gaps provide the strong rationale for assessing synovial fluid profiles early after injury as well as factors associated with the severity of injury.

We hypothesized that information from more acute analyses that incorporate features of the immunologic response and injury characteristics will differentiate patient subsets and provide information that could identify patients that may require anti-inflammatory treatment to augment the standard of care. Therefore, the purposes of this exploratory study were to determine if 1) different inflammatory phenotypes can be determined from the molecular composition of the synovial fluid within 8 d of ACL injury, and 2) patient demographic or injury characteristics differ between inflammatory phenotypes.

## METHODS

This study is a secondary analysis of 41 ACL-injured subjects enrolled at one site as part of a multicenter randomized trial (ClinicalTrials.gov ID: NCT01692756) (10,11). All patients provided informed consent before participating in this IRB-approved trial. The original study was powered to assess the effect of an intraarticular corticosteroid injection versus saline before ACL reconstruction. The current analyses involve only baseline data collected before any treatment being administered. As such, an *a priori* power analysis was not performed for this exploratory secondary analysis.

### Patients

The study included skeletally mature patients between the ages of 14 and 32 that sustained an acute ACL rupture during sports participation. Patients were excluded if they had previous ligamentous or meniscus injury to contralateral knee, a history of previous traumatic ipsilateral knee injury and/or surgery, or clinical evidence of posterior cruciate ligament injury or more than grade 1 medial or lateral collateral ligament injury. Patients were also excluded if the injury occurred more than 8 d before enrollment. Because the original study assessed the effect of a corticosteroid injection, patients were also excluded if they had a known allergy to triamcinolone acetonide, intraarticular cortisone injection into either knee within 3 months of injury, or a history of any inflammatory disease or immunocompromise (11). Patients were not excluded

on the basis of biological sex, race, or concomitant meniscus or articular cartilage injury. Patient demographics were recorded at the baseline visit and pain was assessed on the day of enrollment using a 10 cm Visual Analogue Scale (VAS).

### Severity of Injury

The severity of injury was assessed in three ways: 1) concomitant injuries to the menisci or articular cartilage, 2) the volume of synovial fluid aspirated from the knee at the baseline visit, and 3) the volume of the bone bruises on each patient's preoperative MRI scan. Concomitant injuries to the menisci and articular cartilage were confirmed arthroscopically at the time of surgery. Knee aspiration was performed aseptically within 8 d of injury, and intraarticular placement was confirmed using the "squish" test (18). The knee was aspirated to dryness and the volume of synovial fluid that was aspirated was recorded. The volume of preoperative bone bruises was calculated from each patient's preoperative MRI scan (19). Only patients with MRIs within slice thicknesses <5 mm were included provided T2 or proton density (PD) sequences in the coronal, axial, and sagittal plane were available. Bone bruise volumes were measured from the T2 or PD weighted coronal images using a modified version of Roemer and Bohndorf's technique (19,20). Bone bruise volumes from each of the four bony regions (medial tibial plateau, medial femoral condyle, lateral tibial plateau, and lateral femoral condyle) were then summed and expressed as the total bone bruise volume (mm<sup>3</sup>) (19).

### Biomarker Analyses

Synovial fluid samples were spun for 10 min at 3500 rpm and the supernatant aliquoted and stored at -80°C until analyzed. At the conclusion of the study, samples were shipped to the Duke University Biomarker Shared Resource laboratory for analysis (V.B.K./J.L.H.) (11). Synovial fluid concentrations of cytokines and biomarkers of cartilage degradation, bone turnover, and hemarthrosis were assessed using previously described methods (Table 1) (10,11,15).

**Cytokines.** Proteomic analysis has demonstrated that the cytokine-cytokine receptor pathway is significantly upregulated after acute ACL injury (13), and proinflammatory cytokines IL-1 $\alpha$  and IL-1 $\beta$  and anti-inflammatory IL-1 receptor antagonist (IL-1RA) are pivotal in the pathological processes leading to joint tissue breakdown (21,22). After ACL injury, patients with elevated synovial fluid IL-1 levels have also demonstrated more severe chondral damage (23). IL-1 $\alpha$  has previously been demonstrated to be predictive of patient-reported outcomes after ACL reconstruction (16), and increased expression of IL-1 $\beta$  has been associated with more rapid cartilage degradation among osteoarthritis patients (24). On the contrary, concentrations of the chondroprotective IL-1RA significantly decrease after ACL injury resulting in relatively unopposed activity of IL-1 (25). As such, lower IL-1RA has been associated with more severe chondral damage (23).

For IL-1 $\alpha$  and IL-1 $\beta$  values that were below the lower limit of detection (LLOD), 0.5 LLOD imputed values were used for

TABLE 1. Synovial fluid biomarker concentrations assessed within 8 d of ACL injury (15,16).

Biomarker*	Abbreviation	Volume (Dilution)	CV <sup>a</sup>	LLOD	Number (%) below LLOD
<b>Proinflammatory</b>					
Interleukin-1 $\alpha$ (pg·mL <sup>-1</sup> ) Catalog K151RBD, Meso Scale Discovery, Rockville, MD	IL-1 $\alpha$	25 $\mu$ L (1:2)	9.9%	0.238 pg·mL <sup>-1</sup>	24 (69%)
Interleukin-1 $\beta$ (pg·mL <sup>-1</sup> ) Catalog K151QPD, Meso Scale Discovery, Rockville, MD	IL-1 $\beta$	25 $\mu$ L (1:2)	5.7%	0.033 pg·mL <sup>-1</sup>	16 (46%)
<b>Anti-inflammatory</b>					
Interleukin-1 receptor antagonist (pg·mL <sup>-1</sup> ) Catalog DRA00B, R&D Systems, Minneapolis, MN	IL-1RA	100 $\mu$ L (none)	4.4%	6.3 pg·mL <sup>-1</sup>	0 (0%)
<b>Cartilage degradation</b>					
Sulfated glycosaminoglycan ( $\mu$ g·mL <sup>-1</sup> ) Catalog BP-004, Kamiya Biomedical Company, Seattle, WA	sGAG	25 $\mu$ L (none)	1.8%	N/A	0 (0%)
C-terminal crosslinked telopeptide of type II collagen (ng·mL <sup>-1</sup> ) Cartilaps®, Catalog AC-10F1, Immunodiagnostic Systems, Inc, Fountain Hills, AZ	CTX-II	40 $\mu$ L (none)	4.3%	0.20 $\mu$ g·L <sup>-1</sup>	0 (0%)
Cartilage oligomeric matrix protein ( $\mu$ g·mL <sup>-1</sup> ) Catalog RD194080200, BioVendor, Asheville, NC	COMP	5 $\mu$ L (1:2500)	1.9%	0.4 ng·mL <sup>-1</sup>	0 (0%)
MMP 3 plex (MMP-1, MMP-3, MMP-9) (pg·mL <sup>-1</sup> ) Catalog K15043C, Meso Scale Discovery, Rockville, MD	MMP-1	5 $\mu$ L (1:20)	3.5%	1.74 pg·mL <sup>-1</sup>	0 (0%)
	MMP-3		4.5%	4.34 pg·mL <sup>-1</sup>	0 (0%)
	MMP-9		3.5%	14.3 pg·mL <sup>-1</sup>	0 (0%)
<b>Bone turnover</b>					
N-terminal crosslinked telopeptide of type I collagen (nM BCE) Catalog 9021, Osteomark® NTx; Alere, Scarborough, ME	NTX-I	25 $\mu$ L (1:5)	2.3%	N/A	0 (0%)
<b>Hemarthrosis</b>					
Bilirubin + biliverdin ( $\mu$ mol·L <sup>-1</sup> ) In-house ELISA assay developed by Kraus Laboratory	B + B	30 $\mu$ L (none)	1.3%	1.0 $\mu$ mol·L <sup>-1</sup>	0 (0%)

<sup>a</sup>CV, mean interassay coefficient of variation.

statistical analyses. We also calculated a ratio of IL-1RA to IL-1 $\beta$  to assess the balance of proinflammatory versus anti-inflammatory cytokines. Lower ratios are indicative of a greater imbalance between anti-inflammatory IL-1RA relative to concentration of proinflammatory cytokines.

**Degradative enzymes.** We have demonstrated that proinflammatory stimulation of meniscus cells increases MMP and cytokine activity (26), and the combination of proinflammatory cytokines and compressive loading like what may be seen during sporting and high demand activities further results in degradative enzyme activity and increased production of proinflammatory mediators (27). Similarly after ACL injury, Amano et al. reported significant correlations between MMP-1 and MMP-3 with proinflammatory cytokine concentrations in the synovial fluid (15). In addition, we have previously reported that synovial fluid MMP-9 on the day of ACL reconstruction was significantly greater for those with inferior patient-reported outcomes at minimum 2 yr follow-up (16).

**Biomarkers of cartilage degradation.** Three biomarkers of cartilage degradation were included in this study: sGAG, CTXII, and COMP. Owing to both the mechanical insult of injury and enzymatic degradation, glycosaminoglycans (GAG) content increases immediately after injury (28). Increased synovial fluid concentrations of sGAG at the time of ACL reconstruction were also predictive of worse matrix composition on MRI at 3 yr (15). After cartilage degradation, CTXII is released into the synovial fluid. CTXII has been identified as a biomarker for the diagnosis, staging, and evaluating the prognosis of hip and knee OA (29,30), and has been demonstrated to be responsive over short testing periods (3 months) (31). We have reported that CTXII correlates with the degree of joint destruction and increases significantly within 1 month after ACL injury (10,11). COMP is a marker of cartilage breakdown. Increased COMP has been predictive of joint

space narrowing and osteophyte formation in those with idiopathic knee OA (32). ACL-injured patients have also demonstrated significantly increased COMP when compared to healthy volunteers (33).

**Biomarker of bone turnover.** NTXI is a marker of bone remodeling, and has been shown to be predictive of cartilage thinning for those with idiopathic OA (30). In addition, NTXI has been demonstrated to increase after ACL injury (9).

**Marker of hemarthrosis.** Bilirubin/biliverdin concentration has been used as a biomarker of hemarthrosis and has been shown to be significantly elevated after lower-extremity trauma (34). Bilirubin/biliverdin has also been previously used to quantify hemarthrosis after ACL injury (15).

## Statistical Analyses

The statistical methods utilized in the current study replicate those previously used by Amano et al. (15) Biomarker data were not normally distributed, and consistent with Amano et al., were transformed using Box-Cox transformation (15). A two-cluster analysis was selected to be consistent with Amano et al. but also because of the hypothesis that there may be two underlying clusters of patients based on the 50% prevalence of PTOA within 15 yr after ACL injury (5–7,15).

After performing the cluster analysis, patients were then grouped by cluster assignment. Patient age, BMI, days between injury and enrollment, baseline VAS pain, synovial fluid aspirate volume and bone bruise volumes were compared between clusters using two-tailed independent *t*-tests. Sex and the prevalence of concomitant meniscal and/or articular cartilage injuries were compared between clusters using  $\chi^2$  or Fisher exact tests as appropriate. Biomarker concentrations were compared between clusters using two-tailed independent *t*-tests. All analyses were performed using SPSS

Statistics 24 (IBM, Armonk, NY), and statistical significance was determined with  $P \leq 0.05$ .

**RESULTS**

Of the 41 patients originally enrolled at our site, complete biomarker, imaging, and surgical data were available for 35 patients (85.4%); the MRIs of 6 patients did not meet our standards for analysis. Two clusters were identified (Table 2). The variables that contributed the most to cluster assignment were MMP-1 (ANOVA  $F = 121.4, P < 0.001$ ), MMP-3 ( $F = 13.6, P = 0.001$ ), IL-1RA ( $F = 11.0, P = 0.002$ ), sGAG ( $F = 5.1, P = 0.03$ ), and IL-1 $\beta$  ( $F = 4.7, P = 0.04$ ) (Fig. 1).

One cluster consisting of 10 patients demonstrated significantly greater MMP-1, MMP-3, sGAG, and anti-inflammatory IL-1RA, and significantly lower MMP-9. Although IL-1RA was increased for cluster 1, the ratio of IL-1RA to IL-1 $\beta$  tended to be lower when compared with the other cluster of 25 patients ( $P = 0.053$ , Table 2). Because of the imbalance of proinflammatory versus anti-inflammatory cytokines, we termed this the dysregulated inflammation cluster to be consistent with previous studies of polytrauma patients that used similar terminology (immune dysregulation) (2,3). Patient demographics, bone bruise volumes, synovial fluid aspirate volumes, pain, and concomitant injuries did not differ between the dysregulated inflammation and low inflammation clusters (Table 3).

**DISCUSSION**

The purposes of this study were to determine if 1) inflammatory phenotypes can be determined from the molecular composition of the synovial fluid within 8 d of ACL injury, and 2) if patient demographic or injury characteristics differ between inflammatory phenotypes. Our hypothesis of different patient subsets based on synovial fluid profiling was supported by these results. The most important finding of this study suggests that a subset of patients inherently exhibit a dysregulated inflammatory response after acute injury that is not a function

of injury severity. The current results are similar to a cluster analyses by Namas et al. (2) performed during the first 5 d after blunt polytrauma that identified two patient clusters differing significantly on the basis of multiple inflammatory mediators. Just as in our study, the Namas et al. clusters did not differ in terms of patient demographics or injury severity. Moreover, the proportion of patients within their early immune dysregulation cluster (9 of 33 patients, 27.3%) (2) was very similar to the proportion of patients in the Dysregulated Inflammation cluster in the current study (10 of 35, 28.5%). Collectively, these studies suggest there may be a subset of patients predisposed to a dysregulated inflammatory response after acute injury.

The current results differ somewhat from a previous cluster analysis performed with synovial fluid samples collected on the day of ACL reconstruction (about 6 wk after injury) (15). In this study, they too identified a similar proportion of patients (10 of 26, 38.5%) that fit within an inflammatory cluster with elevated proinflammatory cytokine concentrations, MMP-1, and MMP-3 (15). However, the inflammatory cluster demonstrated significantly lower sGAG concentrations at the time of surgery unlike the Dysregulated Inflammation cluster in our study. Our Dysregulated Inflammation cluster demonstrated significantly increased sGAG approximately 4 d after injury. The reason for this difference is unclear but may be related to the time between injury and sample collection (mean, 4 d vs 64 d after injury); the two studies assessed different periods in the response to injury. Patient age also differed between studies (mean, 19.5 yr vs 34.0 yr). Patterns of cytokines and chemokines differ greatly between elderly trauma patients over the age of 65 when compared with patients younger than 30 yr (35). Patient age differed by only 15 yr between the current study and Amano et al.; it is not known the degree to which this amount of age difference may have impacted the synovial fluid immune profiles.

Although the results of the current study differ slightly from the previous cluster analysis by Amano et al. (15), it is becoming increasingly clear that there is a subset of patients with

TABLE 2. Comparison of synovial fluid biomarker concentrations between the two clusters.

	Dysregulated Inflammation Cluster	Low Inflammation Cluster	<i>P</i> <sup>a</sup>
Cytokine concentrations			
IL-1 $\alpha$ (pg·mL <sup>-1</sup> )	0.92 $\pm$ 2.11	0.32 $\pm$ 0.47	0.39
No. of patients with results < LLOD	5/10 (50%)	19/25 (76%)	0.23
IL-1 $\beta$ (pg·mL <sup>-1</sup> )	1.30 $\pm$ 2.63	0.17 $\pm$ 0.25	0.21
No. of patients with results < LLOD	2/10 (20%)	13/25 (52%)	0.13
IL-1RA (pg·mL <sup>-1</sup> )	<b>15,988 <math>\pm</math> 14,012</b>	<b>4653 <math>\pm</math> 6420</b>	<b>0.03</b>
IL-1RA/IL-1 $\beta$ ratio	49,814 $\pm$ 40,816	126,632 $\pm$ 178,787	0.053
Cartilage degradation			
CTX-II (ng·mL <sup>-1</sup> )	0.74 $\pm$ 0.38	0.84 $\pm$ 0.44	0.54
COMP ( $\mu$ g·mL <sup>-1</sup> )	52.0 $\pm$ 11.6	52.0 $\pm$ 12.3	>0.99
sGAG ( $\mu$ g·mL <sup>-1</sup> )	<b>441.3 <math>\pm</math> 149.5</b>	<b>323.1 <math>\pm</math> 135.9</b>	<b>0.03</b>
MMP-1 (pg·mL <sup>-1</sup> )	<b>1,057,992 <math>\pm</math> 285,749</b>	<b>178,556 <math>\pm</math> 178,658</b>	<b>&lt;0.001</b>
MMP-3 (pg·mL <sup>-1</sup> )	<b>2,551,651 <math>\pm</math> 2,154,629</b>	<b>927,773 <math>\pm</math> 410,839</b>	<b>0.04</b>
MMP-9 (pg·mL <sup>-1</sup> )	<b>13,185 <math>\pm</math> 7792</b>	<b>40,095 <math>\pm</math> 46,222</b>	<b>0.009</b>
Bone turnover			
NTX-I ( $\mu$ g·mL <sup>-1</sup> )	19.7 $\pm$ 9.8	21.2 $\pm$ 6.3	0.68
Hemarthrosis			
Bilirubin+biliverdin ( $\mu$ mol·L <sup>-1</sup> )	32.1 $\pm$ 14.1	31.0 $\pm$ 26.1	0.91

Values are presented as mean  $\pm$  standard deviation or number (%).

Bolded text denotes statistically significant differences between patient clusters.

<sup>a</sup>Raw nontransformed data presented in this table; however, transformed data were used for statistical analyses.

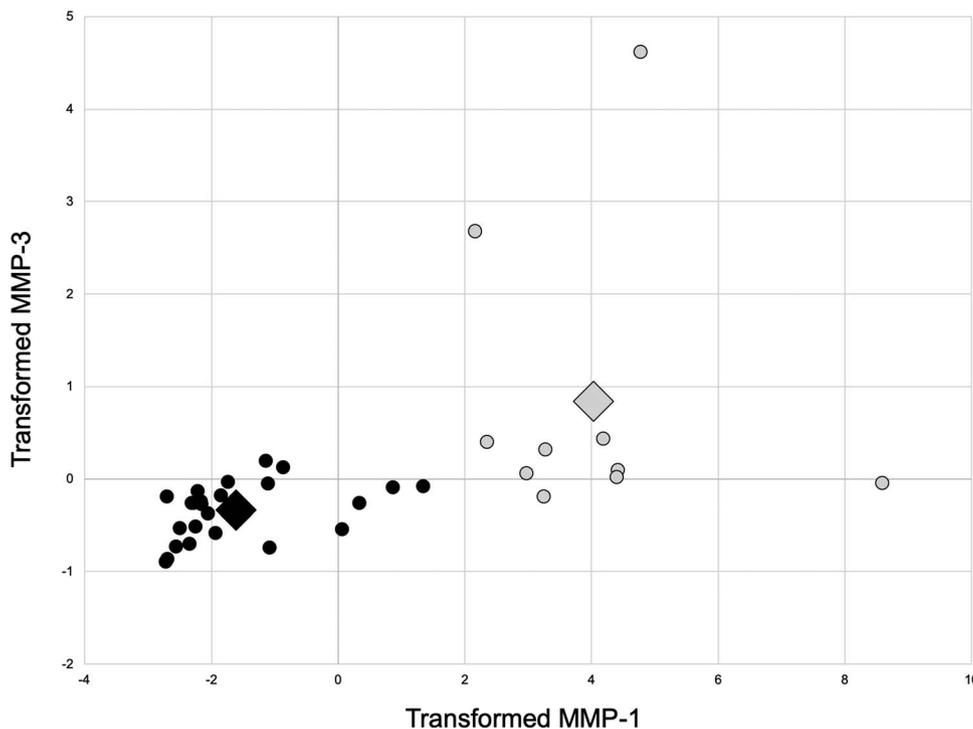


FIGURE 1—Scatterplot of transformed MMP-1 and MMP-3 of the two clusters (gray, dysregulated inflammation cluster; black, low inflammation cluster; large diamond represents the centroid for each cluster).

dysregulated inflammatory responses after acute injury. Not only is there a more robust proinflammatory response, the corresponding innate anti-inflammatory response is muted in this subset of patients. More than 20 yr ago, Dr. Scott Dye described a theory of the knee being “a biological [car] transmission with an envelope of function.” (36) He suggested that the knee is an organ that is not only effected by the treatment provided but also by the complex interplay between anatomic, physiologic, and biomechanical factors. Specific to the current topic, he theorized that there may be phenotypical variations in the response to injury and perhaps genetic predisposition to dysregulation of molecular and/or cellular homeostasis (36). The response to acute injury, whether polytrauma, ACL injury, or meniscus injury, involves a complex dynamic interaction of multiple pathways including the NF-κB, cytokine-cytokine receptor interaction, and osteoclast differentiation pathways, among others (13,37–39). Similar pathways are also implicated in the development and progression of knee osteoarthritis (40–42). Because of the complex nature of this individualized response, a better understanding of the dynamic interplay between multiple pathways may be necessary to improve both short and long outcomes in the future (2,3).

It was intriguing to find that the proportion of patients demonstrating dysregulated inflammatory responses after trauma or ACL injury ranged from 27% to 38%. This suggests that the biologic response to injury does not fully explain the early onset of PTOA after ACL injury as 50% of patients develop PTOA within 15 yr of injury. This leaves a notable proportion of patients that do not exhibit dysregulated inflammatory responses but still progress to PTOA. There are several additional

factors that may contribute to progression to PTOA. Mechanical loading may influence cartilage degradation, and OA patients with a greater cartilage turnover in response to light exercise also demonstrate significantly greater cartilage thinning over the ensuing 2 yr (43). Altered joint mechanics secondary to concomitant injuries treated with chondroplasty or partial meniscectomy can also accelerate the development of OA (44). The chronic proinflammatory effects of altered chondral and meniscal forces were not assessed in this study. In addition, the combination of increased body mass and decreased activity seen in the years after ACL reconstruction (45) may contribute to the progression to PTOA even in those that do not exhibit inflammatory dysregulation immediately after injury. There is also the possibility of reinjury that could account, at least in part, for the “missing” additional incident 25% PTOA. The progression to PTOA is undoubtedly

TABLE 3. Comparison of patient and injury characteristics between the two clusters.<sup>a</sup>

	Dysregulated Inflammation Cluster	Low Inflammation Cluster	P
Demographics			
Age (yr)	21.4 ± 5.1	18.7 ± 3.9	0.15
Sex (F/M; %F)	5/5 (50%)	10/15 (40%)	0.71
BMI (kg·m <sup>-2</sup> )	25.3 ± 4.1	23.4 ± 2.6	0.18
Injury characteristics			
Days after injury	<b>5.6 ± 1.3</b>	<b>3.8 ± 2.2</b>	<b>0.02</b>
Aspirate volume (mL)	34.2 ± 25.8	22.0 ± 16.4	0.10
Bone bruise volume (mm <sup>3</sup> )	14.6 ± 19.6	8.3 ± 7.7	0.18
VAS pain	3.4 ± 3.1	5.5 ± 2.9	0.08
Medial meniscus tear	5/10 (50%)	20/25 (80%)	0.11
Lateral meniscus tear	7/10 (70%)	16/25 (64%)	>0.99
Articular cartilage lesion	1/10 (10%)	0/25 (0%)	0.29

<sup>a</sup>Bold, italics denotes statistically significant differences between patient clusters.

multifactorial, further justifying the need to assess personalized signatures after injury to alter the path of this complex condition.

This study was not without limitation. First, it represents pilot work to assess the synovial fluid profiles of 35 patients immediately after ACL injury; the results should be considered discovery based for future hypothesis generation. There may be the potential for additional clusters or patient phenotypes that could be identified with a larger sample size. Second, our study was focused on IL-1, whereas others have suggested assessing a wider spectrum of both proinflammatory and anti-inflammatory cytokines (2,4,15). Additionally, even at a mean of 4 d after injury, a large number of samples had IL-1 $\alpha$  and IL-1 $\beta$  values below the lower limits of detection, especially in the Low Inflammation cluster. Finally, we used very narrow inclusion–exclusion criteria, and additional studies will be necessary to determine if these results are generalizable to older age groups and those with multiple ligament injuries or other acute knee injuries.

In conclusion, increased cartilage breakdown and degradative enzyme activity after ACL injury may be related to a dysregulated inflammatory response demonstrated by a subset of

patients and do not appear to be a function of injury severity. We identified a cluster of patients with a dysfunctional inflammatory response that also demonstrated significantly greater concentrations of biomarkers previously associated with PTOA progression, namely sGAG, MMP-1, and MMP-3 (15,46). Future studies are necessary to determine if 1) early anti-inflammatory treatment may alter the cycle of persistent inflammation and cartilage degradation in this subset of patients, and 2) the timing of surgery should be earlier to avoid the “second-hit” of inflammation associated with surgery or delayed until after the initial inflammatory response has subsided.

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Results of the present study do not constitute endorsement by the American College of Sports Medicine, and the authors declare no conflicts of interest. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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