Distinct Pattern of Inflammation of Articular Cartilage and the Synovium in Early and Late Hip Femoroacetabular Impingement

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Background: The molecular mechanism of how femoroacetabular impingement (FAI) morphology leads to hip osteoarthritis (OA) is yet to be determined. The expression and location of inflammation-related molecules during early- and late-stage FAI have not been previously described. Moreover, the characterization of intra-articular inflammation away from the cam deformity as well as the nature of adjacent synovial tissue have also not been extensively reported.

Hypothesis: Early-stage FAI has a similar expression of inflammation-related markers in the head-neck and acetabular cartilage but less synovitis than late-stage FAI.

Study Design: Controlled laboratory study.

Methods: Head-neck cartilage, acetabular cartilage, and synovial samples were obtained from patients undergoing hip preservation surgery for the treatment of symptomatic cam FAI (early FAI group; n = 15) and advanced OA secondary to cam FAI (late FAI group; n = 15). Samples procured from healthy young adult donors served as the control group (n = 7). Cartilage degeneration was assessed by histology, and the expression of inflammation-related proteins (interleukin–1 beta [IL-1β], matrix metalloproteinase–13 [MMP-13], a disintegrin and metalloproteinase with thrombospondin motifs–4 [ADAMTS-4], type II collagen [COL2], and aggrecan neoepitope [NITEGE]) was measured by immunostaining. Synovial samples in the early and late FAI groups were examined for synovitis and the expression of IL-1β.

Results: Head-neck cartilage in the early FAI group showed significantly more degeneration than the control group and an increased expression of inflammation-related proteins (IL-1β: 69.7% ± 18.1% vs 20.2% ± 4.9%, respectively; MMP-13: 79.6% ± 12.6% vs 25.3% ± 9.5%; ADAMTS-4: 83.9% ± 12.2% vs 24.3% ± 11.1%; NITEGE: 89.7% ± 7.7% vs 39.8% ± 20.5%) ($P < .001$). Head-neck and acetabular cartilage in the early and late FAI groups showed a similar degree of degeneration. Moreover, a similar expression of inflammation-related proteins was observed between the early and late FAI groups for head-neck cartilage (IL-1β: 69.7% ± 18.1% vs 72.5% ± 13.2%; MMP-13: 79.6% ± 12.6% vs 71.4% ± 18.8%; ADAMTS-4: 83.9% ± 12.2% vs 82.6% ± 12.5%; COL2: 93.6% ± 3.9% vs 92.5% ± 5.8%; NITEGE: 89.7% ± 7.7% vs 95.7% ± 4.7%) and acetabular cartilage (IL-1β: 83.3% ± 24.8% vs 80.7% ± 15.6%; MMP-13: 94.3% ± 9.7% vs 85.2% ± 12.3%; ADAMTS-4: 98.5% ± 2.3% vs 98.4% ± 3.4%; COL2: 99.8% ± 0.7% vs 99.7% ± 1.1%; NITEGE: 96.7% ± 6.7% vs 99.2% ± 2.2%). In contrast, synovitis was minimal with a low expression of IL-1β in the early FAI group compared with the late FAI group.

Conclusion: Hip cartilage exhibited an OA phenotype in patients with early-stage FAI, similar to what was observed in hip OA secondary to FAI. Severe synovitis was only evident with late-stage FAI.

Clinical Relevance: This study supports the concept that early hip impingement is associated with cartilage degeneration and catabolism.

Keywords: osteoarthritis; femoroacetabular impingement; cartilage degeneration; synovitis

Osteoarthritis (OA) is a common and debilitating disease affecting 30 million adults in the United States.[5] Femoroacetabular impingement (FAI) has been identified as a major cause of hip pain in young adult patients and a potential generator of secondary OA in up to 50% of hip OA cases.[31] In cross-sectional and longitudinal studies of hip OA, cam FAI has consistently shown an association with developing hip OA.[39] Recently, local inflammation of the synovium, cartilage, and subchondral bone has been shown to contribute to OA progression through the

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production of inflammatory cytokines and matrix proteases.[14] FAI is associated with local molecular inflammation,[6,10,13,17] which likely leads to hip OA.

Interleukin–1 beta (IL-1β) is one of the main cytokines involved in pathogenesis during the progression of OA,[15] as it contributes to articular cartilage destruction by promoting the production of various proteinases including extracellular matrix (ECM)–degrading enzymes such as matrix metalloproteinase–13 (MMP-13) and a disintegrin and metalloproteinase with thrombospondin motifs–4 (ADAMTS-4).[2,24] Type II collagen (COL2) and aggrecan are main components of the ECM in cartilage.[30] During the progression of OA, the ECM of cartilage is actively remodeled by chondrocytes under inflammatory conditions.[21] The molecular mechanism of how FAI morphology leads to OA is yet to be determined, and the expression and location of the aforementioned molecules during early- and late-stage FAI have not been previously described. The characterization of intra-articular inflammation away from the cam deformity as well as the nature of adjacent synovial tissue have also not been extensively reported. Previously, we have observed an OA phenotype with a high expression of inflammation-related markers in cartilage from the head-neck area in patients with symptomatic cam FAI.[16] Additionally, Abrams et al[1] reported that patients with FAI showed baseline levels of synovial inflammation and that synovitis scores were increased in patients with cartilage damage.

The goals of this study were (1) to characterize the levels of inflammation-related markers in head-neck cartilage, acetabular cartilage, and the synovium from patients with symptomatic cam FAI and (2) to compare these markers to those of end-stage disease. We hypothesized that early-stage cam FAI would have a similar expression of inflammation-related markers in the head-neck and acetabular cartilage at the impingement zone compared with late-stage cam FAI but less synovitis than late-stage cam FAI.

METHODS

Patients and Tissue Collection

This study was approved by the institutional review board at Washington University in St Louis (No. 201703054), and informed consent was obtained from all patients. The diagnosis of cam FAI or OA was determined by the treating surgeons using the criteria of pain in the affected hip for a period of >3 months; hip range of motion including the flexion, adduction, internal rotation (FADIR) test; and radiographic and intraoperative findings. The Tönnis classification[35] was used to define the severity of OA (no OA or pre-OA: grade 0 or 1; advanced OA: grade 2 or 3), and a cam deformity was defined by an alpha angle of >55° on preoperative anteroposterior (AP) pelvis, frog lateral, and/or 45° Dunn lateral radiographs.[9] On preoperative AP pelvis radiographs, developmental dysplasia of the hip (DDH) was defined by a lateral center-edge angle (LCEA) of <20°, and a pincer deformity was defined by an LCEA of >40°.[19] High intraobserver and interobserver reliability of the radiographic analysis of hip morphology has been previously reported.[25]

All surgical procedures were performed between 2017 and 2018. Head-neck cartilage, acetabular cartilage, and synovial samples were harvested using an arthroscopic biter or half-inch osteotome in patients undergoing hip preservation surgery for the treatment of symptomatic cam FAI (early FAI group; n = 15) and patients undergoing total hip replacement for the treatment of advanced OA secondary to cam FAI (late FAI group; n = 15), as shown in Figure 1. All head-neck cartilage samples were obtained from the anterolateral aspect of the head-neck junction (impingement area). Acetabular cartilage samples were obtained from the worst macroscopic lesion in the acetabular rim. Synovial samples were obtained from the worst macroscopic area. Exclusion criteria included previous surgery, pincer morphology, DDH, OA with excessive osteophytes, infections, osteonecrosis of the femoral head, psoriasis, and rheumatological conditions. As a control group, cartilage samples were harvested from the anterolateral head-neck area of fresh femoral head allografts from healthy young adult donors (n = 7). Allografts were obtained within 24 hours of donor death (JRF Ortho) and delivered to the laboratory in a cell culture medium at 4°C. The study design is summarized in Table 1.

Histological Evaluation

Cartilage and synovial specimens were fixed in 10% neutral buffered formalin for 24 to 48 hours, dehydrated, embedded in paraffin wax, and sectioned (5 μm). Cartilage sections were stained with safranin-O to analyze histological features and to estimate the content and distribution

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TABLE 1
Study Design[a]

	Control (n = 7)	Early FAI (n = 15)	Late FAI (n = 15)
OA phenotype of cartilage	Safranin-O/Mankin score	Safranin-O/Mankin score	Safranin-O/Mankin score
Chondrocyte viability	TUNEL assay	TUNEL assay	TUNEL assay
Protein expression in cartilage (IHC)	IL-1β, MMP-13, ADAMTS-4, COL2, NITEGE	IL-1β, MMP-13, ADAMTS-4, COL2, NITEGE	IL-1β, MMP-13, ADAMTS-4, COL2, NITEGE
Synovitis (No. of samples)	NA	H&E staining/synovitis score (early FAI: n = 13; late FAI: n = 14)	H&E staining/synovitis score (early FAI: n = 13; late FAI: n = 14)
Protein expression in synovium (IHC)	NA	IL-1β	IL-1β

[a]ADAMTS-4, a disintegrin and metalloproteinase with thrombospondin motifs–4; COL2, type II collagen; FAI, femoroacetabular impingement; H&E, hematoxylin and eosin; IHC, immunohistochemistry; IL-1β, interleukin–1 beta; MMP-13, matrix metalloproteinase–13; NA, not available; NITEGE, aggrecan neoepitope; OA, osteoarthritis; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

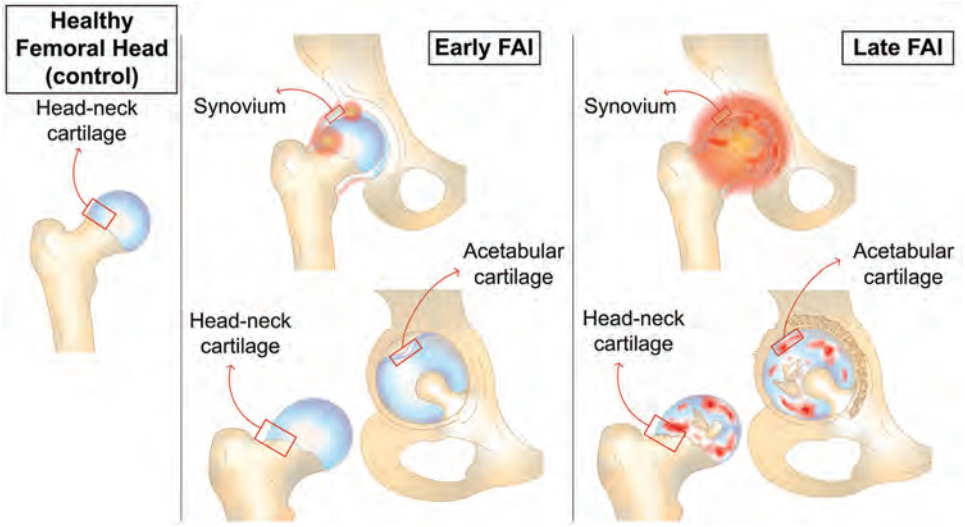

Figure 1. Images showing the locations of sample collection (red boxes). FAI, femoroacetabular impingement.

of proteoglycans. Cartilage degeneration was graded based on the Mankin score[22] in a blinded fashion by 3 investigators (M.H., L.C., and C.P.-G.). The severity of OA using the Mankin score was defined in a previous study (0: normal; 1-4: mild; 5-9: moderate; 10-14: severe).[12] Intraobserver and interobserver reliability of the Mankin score has previously been shown to be adequate.[36] In this study, the intraclass correlation coefficient of the Mankin score among 3 observers (M.H., L.C., and C.P.-G.) was 0.8912. Synovial sections were stained with hematoxylin and eosin (H&E) and were evaluated for synovitis using a histopathological scoring system (0-1: no synovitis; 2-4: low-grade synovitis; 5-9: high-grade synovitis).[20]

Immunohistochemistry

Slides were deparaffinized, rehydrated, and digested with 10 μg/mL proteinase K for antigen retrieval. After blocking with 10% goat serum, slides were incubated with the following primary antibodies procured from Abcam at 1:100 dilutions at 4°C overnight: rabbit anti–IL-1β (ab2105), rabbit anti–MMP-13 (ab39012), rabbit anti–ADAMTS-4 (ab185722), rabbit anti-COL2 (ab34712), and rabbit anti-NITEGE (aggrecan neoepitope) (in house). Immunostaining for anti-NITEGE, an aggrecan neoepitope antibody,[11] was performed to detect the C-terminal site of the cleaved aggrecan and demonstrate the effect of increased metalloproteinase activities. Slides were incubated with a secondary antibody (ab6721; Abcam) at a 1:1000 dilution for 2 hours. A signal was developed using a peroxidase substrate product (Betazoid DAB Chromogen Kit; Biocare Medical). Hematoxylin was used as a counterstain. Sections were analyzed using a NanoZoomer scanner (Hamamatsu Photonics). The percentage of immunopositive cells from the total cell count on the cartilage sections was calculated using ImageJ (National Institutes of Health)[32] in a blinded fashion by the 3 observers. Cells above the tidemark were included in the analysis.

Assessment of Chondrocyte Viability

A cell viability test was performed to confirm that the difference in the expression of inflammation-related markers was not related to a loss of cell viability. The viability of

TABLE 2
Characteristics of Study Participants[a]

	Control (n = 7)	Early FAI (n = 15)	Late FAI (n = 15)	*P* Value
Age, y	25.1 ± 7.1	32.5 ± 9.0	57.1 ± 7.5	<.001
Sex, male/female, n	2/5	11/4	13/2	.3613
Body mass index, kg/m^2	NA	28.1 ± 4.7	31.4 ± 6.5	.3232
Tönnis classification	NA	0.5 ± 0.5	3.0 ± 0.0	<.001
Alpha angle, deg	NA	70.1 ± 11.6	84.2 ± 12.1	.0084
LCEA, deg	NA	28.0 ± 6.0	28.4 ± 8.8	.8682

[a]Data are shown as mean ± SD unless otherwise indicated. FAI, femoroacetabular impingement; LCEA, lateral center-edge angle; NA, not available.

chondrocytes was assessed by in situ detection of chondrocyte apoptosis using a TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay (Roche). Images were acquired using a DMi8 confocal laser scanning microscope (Leica). The cellular viability percentage was assessed using ImageJ.[32]

Statistical Analysis

The Kruskal-Wallis test, followed by the Steel-Dwass post hoc test, was applied for multiple comparisons. The Mann-Whitney *U* test or chi-square test was used for comparisons between 2 groups. $P < .05$ indicated statistically significant differences. All values are presented as the mean ± SD. Data analysis was performed with JMP 14 (SAS Institute).

RESULTS

Patients' Characteristics

The characteristics of study participants are presented in Table 2. The mean alpha angle in the early (70.1° ± 11.6°) and late (84.2° ± 12.1°) FAI groups was higher than 55°, confirming a cam deformity. The mean LCEA in the early and late FAI groups was 28.0° ± 6.0° and 28.4° ± 8.8°, respectively, ruling out concomitant hip dysplasia. No difference was seen in body mass index (P = .3232) and sex (P = .3613) between the early and late FAI groups.

Histological Findings

Head-Neck Cartilage. There was no difference in head-neck cartilage between the early and late FAI groups, with moderate cartilage degeneration (P = .3967), but both differed from the control group (Mankin score for control: 1.6 ± 1.3; early FAI: 8.3 ± 2.1; late FAI: 7.1 ± 1.9) ($P < .001$) (Figure 2, A and B). Compared with the control group, head-neck cartilage in the early and late FAI groups highly expressed inflammatory and catabolic markers: IL-1β (20.2% ± 4.9%, 69.7% ± 18.1%, and 72.5% ± 13.2%, respectively), MMP-13 (25.3% ± 9.5%, 79.6% ± 12.6%, and 71.4% ± 18.8%, respectively), ADAMTS-4 (24.3% ± 11.1%, 83.9% ± 12.2%, and 82.6% ± 12.5%, respectively), and NITEGE (39.8% ± 20.5%, 89.7% ± 7.7%, and 95.7% ± 4.7%, respectively) ($P < .001$). The expression of COL2 was similar among the groups (control: 95.4% ± 6.4%; early FAI: 93.6% ± 3.9%; late FAI: 92.5% ± 5.8%) (P = .3707) (Figure 2, C and D). Head-neck cartilage in all groups showed similar viability (control: 93.5% ± 2.7%; early FAI: 96.2% ± 4.0%; late FAI: 93.8% ± 4.4%) (P = .5866) (Figure 2, E and F).

Acetabular Cartilage. The early and late FAI groups demonstrated degeneration of the acetabular cartilage. Cartilage degeneration was worst in the early FAI group (Figure 3A). Specifically, severe loss of the ECM was observed (Mankin score for early FAI: 9.3 ± 1.4; late FAI: 7.0 ± 0.8) ($P < .001$) (Figure 3B). Acetabular cartilage in both the early and late FAI groups showed a similar expression of inflammatory and catabolic proteins: IL-1β (83.3% ± 24.8% and 80.7% ± 15.6%, respectively; P = .3086), MMP-13 (94.3% ± 9.7% and 85.2% ± 12.3%, respectively; P = .0640), ADAMTS-4 (98.5% ± 2.3% and 98.4% ± 3.4%, respectively; P = .5696), and NITEGE (96.7% ± 6.7% and 99.2% ± 2.2%, respectively; P = .1469). The expression of COL2 was similar between the 2 groups (early FAI: 99.8% ± 0.7%; late FAI: 99.7% ± 1.1%) (P = .8036) (Figure 3, C and D). Both groups showed similar viability (early FAI: 97.3% ± 1.3%; late FAI: 96.2% ± 1.5%) (P = .6650) (Figure 3, E and F).

Synovitis. Synovitis was minimal in the early FAI group but severe in the late FAI group (Figure 4A). In the early FAI group, most samples showed low-grade (n = 8; 61.5%) or no synovitis (n = 4; 30.8%). Only 1 sample (7.7%) had high-grade synovitis. In the late FAI group, however, 6 of 14 samples (42.9%) showed high-grade synovitis, 7 samples (50.0%) showed low-grade synovitis, and only 1 (7.1%) had no synovitis. The mean synovitis score was lower in the early FAI group than the late FAI group (2.5 ± 1.7 and 4.4 ± 1.6, respectively) (P = .0121) (Figure 4B). Additionally, a lower IL-1β expression was observed in the early FAI group compared with the late FAI group (Figure 4C).

DISCUSSION

Head-neck and acetabular cartilage from the impingement zone of hips with early- and late-stage FAI showed moderate cartilage degeneration and markedly elevated levels of selected inflammatory and catabolic markers compared

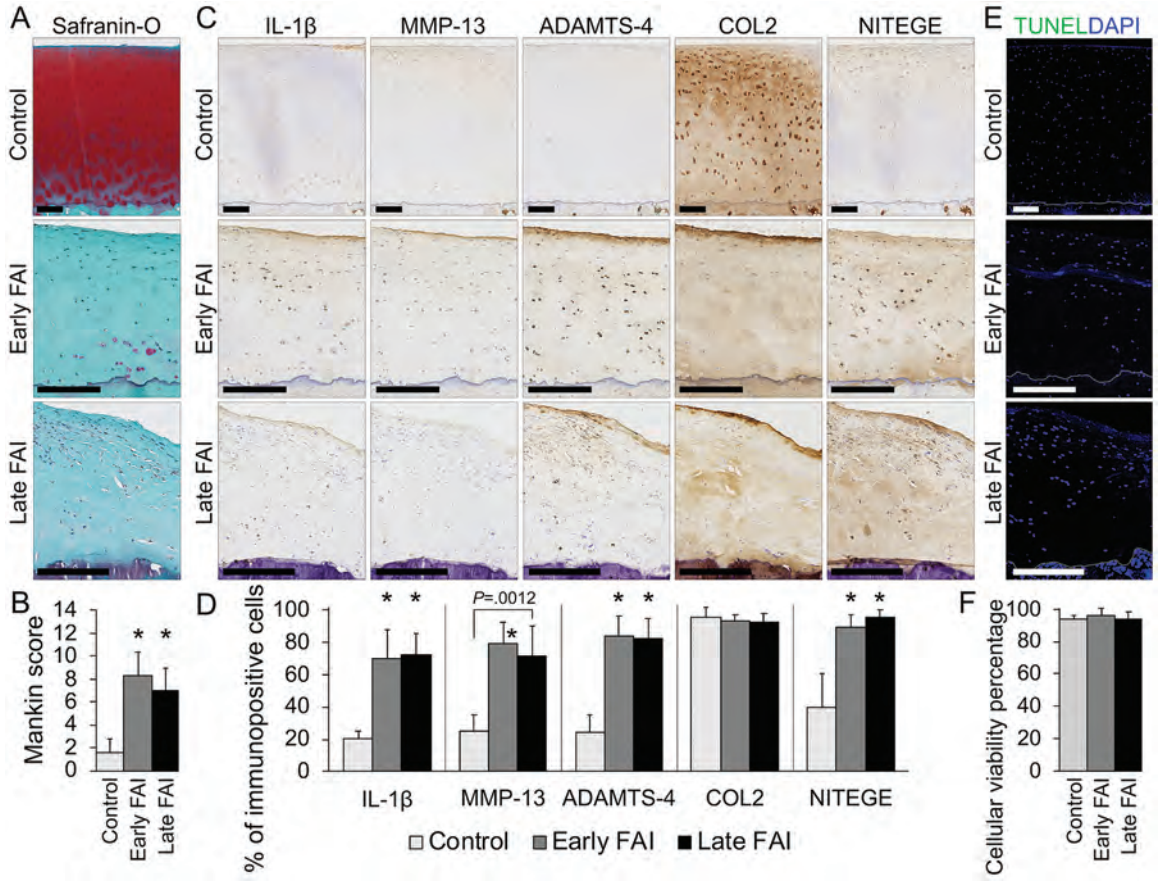

Figure 2. Histological assessment of head-neck cartilage in the control, early femoroacetabular impingement (FAI), and late FAI groups. (A) Safranin-O staining. (B) Mankin score. (C) Immunostaining for IL-1β, MMP-13, ADAMTS-4, COL2, and NITEGE. (D) Percentage of immunopositive cells. (E) TUNEL assay. (F) Cellular viability percentage. Scale bar = 250 μm. *Significant differences compared with the control group ($P < .001$). ADAMTS-4, a disintegrin and metalloproteinase with thrombospondin motifs–4; COL2, type II collagen; IL-1b, interleukin–1 beta; MMP-13, matrix metalloproteinase–13; NITEGE, aggrecan neoepitope; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

with normal head-neck cartilage. Late-stage FAI was associated with greater synovitis and a higher synovial expression of IL-1β compared with early-stage FAI.

Microscopic degenerative OA changes in head-neck and acetabular cartilage have been reported previously.[18,23,28,33,37] The current study is distinct and among the first published because it includes cartilage from the head-neck and acetabulum as well as the synovium from patients with early-stage FAI, patients with late-stage OA secondary to FAI, and healthy controls. Moreover, all samples were characterized with H&E staining, safranin-O staining, and immunostaining for inflammation-related markers. Finally, the cellular viability of cartilage was assessed to confirm that immunonegative findings were not related to a loss of cellular viability.

In our study, we investigated inflammatory and catabolic markers with immunostaining to locate these markers within cartilage tissue. The expression of inflammatory and catabolic markers was similar in early- and late-stage FAI. Our immunohistochemical findings in head-neck and acetabular cartilage are similar to those of Hashimoto et al,[17] who reported that no differences were observed in the mRNA expression of *IL-1β, MMP-13*, and *COL2A1* between early- and late-stage FAI. We found that the expression of COL2 was similar among all groups. Previously, Rai et al[27] reported that the mRNA expression of *COL2A1* was up-regulated in more severely damaged OA cartilage of the knee compared with moderate degenerated cartilage. These findings suggest that chondrocytes respond anabolically to inflammation, with reinitiation of the synthesis of the ECM, specifically COL2,[30] in an attempt to repair the damaged matrix.[29] On the other hand, Chinzei et al[6] reported that head-neck cartilage showed a higher mRNA expression of *IL-1β, MMP-13*, and *ADAMTS-4* in the early-stage FAI group compared with the OA group. Chinzei et al's study did not report if OA was secondary to FAI, and most of the patients with OA were female, suggesting that their population was not similar to ours. In our study, 86.7% of patients with OA were male because we only included patients who had OA secondary to hip FAI (cam deformity). We would not expect similar findings in primary OA or OA secondary

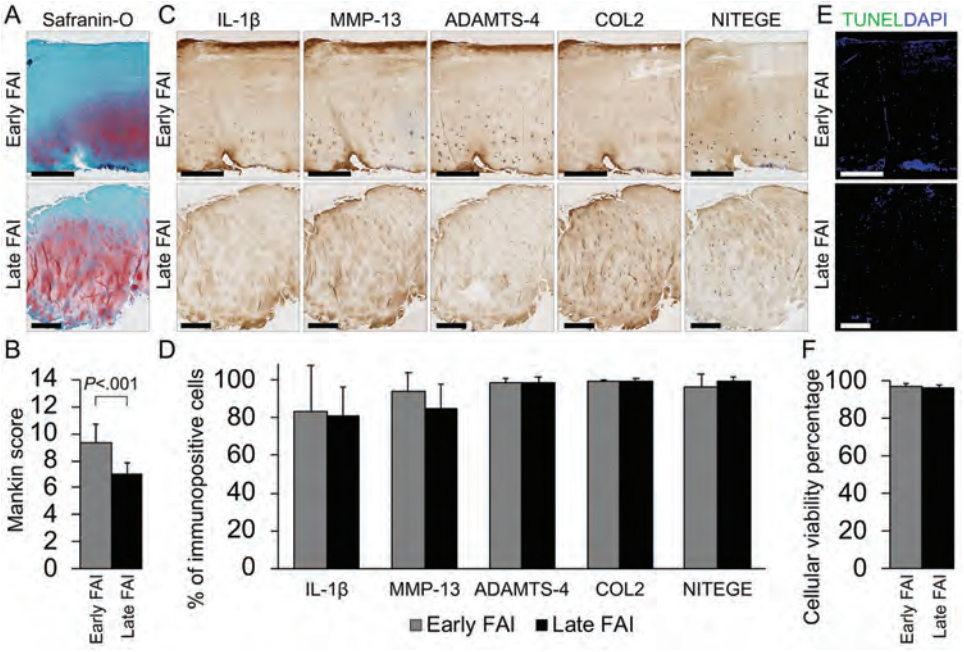

Figure 3. Histological assessment of acetabular cartilage in the early femoroacetabular impingement (FAI) and late FAI groups. (A) Safranin-O staining. (B) Mankin score. (C) Immunostaining for IL-1β, MMP-13, ADAMTS-4, COL2, and NITEGE. (D) Percentage of immunopositive cells. (E) TUNEL assay. (F) Cellular viability percentage. Scale bar = 500 μm. ADAMTS-4, a disintegrin and metalloproteinase with thrombospondin motifs–4; COL2, type II collagen; IL-1b, interleukin–1 beta; MMP-13, matrix metalloproteinase–13; NITEGE, aggrecan neoepitope; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

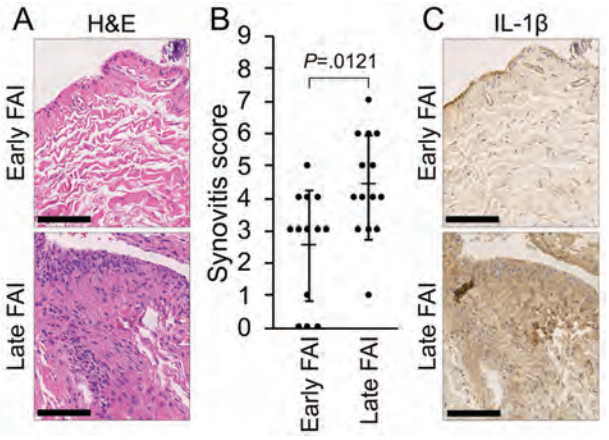

Figure 4. Assessment of synovitis in the early femoroacetabular impingement (FAI) and late FAI groups. (A) Hematoxylin and eosin (H&E) staining. (B) Synovitis score. (C) Immunostaining for IL-1β (Interleukin–1 beta). Scale bar = 125 μm.

to DDH, as the head-neck would not be under chronic impingement. Previous studies have proved that the severity of a cam deformity is worse in male patients, with higher alpha angles and more severe types of impingement.[26] This metabolic hyperactivity observed in cartilage from patients with early-stage FAI suggests that inflammation and degeneration commonly observed in patients with OA have already occurred in early-stage FAI.

In addition, the cellular viability in head-neck cartilage was similar among all groups. Also, no difference of cellular viability in acetabular cartilage from the early and late FAI groups was observed. These findings suggest that the pattern of expression of selected inflammatory and catabolic markers was not related to a loss of cell viability but to the state of cellular activity, that is, whether cells are producing these proteins. Similarly, previous studies have shown that high chondrocyte viability (87%-95%) was confirmed in delaminated acetabular cartilage[4,23,38] and more than 95% viability in damaged femoral head-neck cartilage in patients with FAI.[4]

Interestingly, there was less synovitis in the early FAI group compared with the late FAI group. Because synovial tissue is not located at the zone of impingement, it does not receive the mechanical stresses directly from hip impingement, probably resulting in less inflammation or a slower progression of disease than that observed in cartilage from the impingement zone. In a study by Abrams et al,[1] synovial samples harvested from 12 patients with FAI histologically demonstrated baseline synovitis. Also, Chinzei et al[6] reported that the mRNA expression of *IL-1β* in synovial samples from patients with FAI was lower than in OA samples. Synovial tissue from OA typically displays mild/moderate synovitis.[8] To our knowledge, this is the first study to compare histological synovitis between patients with cam FAI and patients with advanced hip OA secondary to FAI. However, our data on synovial samples are limited because we only took 1 sample from the worst macroscopic area, limiting the investigation of the status

of intra-articular synovial inflammation in hip FAI. Furthermore, Talks et al[34] reported that no systemic inflammation, defined by the serum-free light chain concentration in serum samples, was found in patients with symptomatic FAI, suggesting that FAI is localized. However, Bedi et al[3] demonstrated that, compared with age- and activity-matched athletes without FAI, those with FAI had a 24% increase in mean circulating levels of cartilage oligomeric matrix protein (COMP) and a nearly 3-fold elevation in mean circulating levels of C-reactive protein (CRP). COMP has been used as a marker of cartilage turnover in patients with OA, and CRP has been used as a marker of inflammation in OA and many other chronic conditions.[7] Additionally, biopsy specimens of tissue from patients undergoing FAI corrective surgery have shown clear signs of local inflammation in the hip joint.[6,10,17] Further studies assessing synovial tissue from different intra-articular areas will be critical to investigate the role of synovitis in hip FAI.

This study has a number of limitations. First, diseased cartilage samples were taken only from symptomatic patients who underwent surgical treatment. It is not ethically appropriate to collect samples from patients who have been diagnosed with FAI but have no symptoms. Second, we were unable to obtain normal acetabular cartilage and synovial tissue from healthy young adult donors, although normal head-neck cartilage was used as a control. Third, the age of the early and late FAI groups could not be matched. Previous studies have observed no differences in gene expression related to inflammation when comparing head-neck cartilage between young ($<$30 years) and old ($\geq$30 years) patients.[6] Fourth, in late-stage FAI, an increase in the alpha angle may be caused by osteophytes present in OA. To reduce this possibility, patients who had an LCEA of $>40^\circ$, severe deformities, or excessive osteophytes were not included. Finally, to thoroughly assess the state of intra-articular inflammation at different stages of disease, several samples should be harvested from different intra-articular locations, not only the affected head-neck and acetabulum areas. However, this approach would not be ethically appropriate.

Despite these limitations, the current study demonstrated similar levels of molecular inflammation in articular cartilage of the head-neck and acetabulum during early- and late-stage FAI. Late-stage FAI was associated with greater synovitis than early-stage FAI. This may be an important mechanism by which repeated hip impingement causes chronic inflammation. How the differential inflammation that we observed in the synovium from early- and late-stage disease contributes to either the symptoms or progression of OA requires further study.

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