Knee Osteotomy Decreases Joint Inflammation Based on Synovial Histology and Synovial Fluid Analysis

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Purpose: To examine the biological changes in the joints of patients with knee osteoarthritis (OA) before and after around-knee osteotomy (AKO), focusing on synovial fluid (SF) and synovial pathological changes. **Methods:** Patients who underwent AKO for medial compartment knee OA between 2019 and 2021 were examined. SF and synovium were obtained at the time of AKO and plate removal after bone union (mean, 16.8 months [range: 11−38 months] postoperatively). SF volume and interleukin (IL)-6 concentrations in SF were assayed using enzyme-linked immunosorbent assay. Synovitis was assessed histologically using a semiquantitative scoring system. Macrophage infiltration was assessed by immunohistochemistry using a semiquantitative score for F4/80 expression. The M1/M2 ratio was calculated using percentage of cells positive for CD80 and CD163. The expression of proinflammatory cytokines was assessed by the percentage of IL-1β- and IL-6-positive cells. The number of vascular endothelial growth factor-positive luminal structures was counted to assess angiogenesis. The change in each parameter was compared before and after AKO using the Wilcoxon matched-pairs signed-rank test. **Results:** Twenty-four knees of 21 patients were included. SF volume and IL-6 concentration significantly decreased postoperatively (12.6 ± 2.1 mL vs 4.2 ± 0.6 mL; $P < .0001$ and 50.5 ± 8.6 pg/mL vs 20.7 ± 3.8 pg/mL; $P = .0001$, respectively). A significant reduction in synovitis score ($P = .0001$), macrophage infiltration ($P < .0003$), M1/M2 ratio ($P < .0007$), angiogenesis ($P < .0001$), and the percentage of IL-1β- and IL-6-positive cells in the intima ($P < .008$ and $P < .002$, respectively) was found after AKO. **Conclusions:** SF volume and IL-6 concentrations in the SF decreased and inflammatory synovium pathology improved after AKO. In addition to biomechanical changes, the biological environment of the joint can be improved after AKO. **Level of Evidence:** Level IV, retrospective therapeutic case series.

See commentary on page 844

Osteoarthritis (OA) is the most common form of joint disease, characterized by cartilage wear. Genetic predisposing background, excessive mechanical loading, and biological factors have been suggested to be associated with OA.[1] During OA progression, a variety of biological changes occur, including subchondral bone change, osteophyte formation, and synovitis. In recent years, a growing number of reports have suggested that synovitis is one of the major pathological conditions of OA progression.[2−6]

The synovium consists of two layers: intima and subintima. The intima is a superficial layer that faces the joint cavity and contains macrophages and fibroblasts. The subintima is the layer under the intima that contains vascular and lymphatic vessels, smooth muscle cells, and other resident cells.[7] In OA synovium,

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hyperplasia of the intimal layer, infiltration of inflammatory cells into the subintimal layer, hypervascularization, and presence of osteochondral fragments have been reported.[8] Previous studies have implicated that macrophages play a major role in synovial and joint inflammation in association with phenotypic changes toward inflammatory M1 from the homeostatic M2 phenotype.[9,10] In addition, distinct macrophage populations in the synovium, characterized by the expression of CX_3CR1, a chemokine receptor, play different roles during the development of arthritis. Therefore, OA is closely associated with synovial macrophages. Thus, a better understanding of the pathological conditions of the synovium, including macrophages, appears to be an important step toward improving the treatment of OA.[8,11,12]

In patients with OA, joint effusion is frequently associated with OA progression. Joint effusion is considered to be an indicator of the inflammatory condition of the joint.[13] A variety of inflammatory cytokines were found in the joint fluid of patients with OA, and previous studies have reported that interleukin (IL)-6 concentration was higher in the synovial fluid (SF) of patients with knee OA.[14–18] It has also been reported that IL-6 levels in SF correlates with pain.[16] Synovitis is recognized as an important pathological condition in the development and progression of OA.

Around-knee osteotomy (AKO), such as high tibial osteotomy (HTO), distal femoral osteotomy, and double-level osteotomy (DLO), is a surgical method for the treatment of knee OA with abnormal limb mechanical alignment.[19,20] Good clinical outcomes have been reported for various types of AKO.[21,22] The main aim of AKO is to reduce joint loading in the affected medial or lateral compartment of the knee. The beneficial effects of AKO on cartilage and bone were suggested by the evidence of regeneration of cartilaginous tissue after AKO.[23] Although the biomechanical effects of AKO have been well examined and confirmed in previous reports,[24–29] biological changes in the joint, particularly in the synovium, before and after AKO remain unclear. Therefore, the purpose of this study was to examine the biological changes in the joints of patients with knee OA before and after AKO, focusing on synovial fluid (SF) and synovial pathological changes. The hypothesis was that SF volume and IL-6 concentration in SF would decrease and that synovium inflammation would improve in association with improvement in synovial angiogenesis, inflammatory status of macrophage, and synovial structure after AKO.

Methods

This study was approved by the Institutional Review Board of Kobe University (ID no. B190030).

Subjects

This retrospective analysis of prospectively collected data included patients who underwent AKO for medial knee OA between 2019 and 2021 at one institution. The surgical indications were as follows: patients aged <80 years with relatively high activity; OA lesions limited to the medial compartment, as confirmed radiographically; and no injury or instability of the anterior or posterior cruciate ligaments as confirmed by manual examination and magnetic resonance imaging (MRI). The contraindications were as follows: presence of concomitant inflammatory disease (e.g., rheumatoid arthritis [RA]), flexion contracture of more than 20°, infectious arthritis, and history of immunosuppressive therapy (e.g., steroids). Patients with severe OA associated with bone defects were also contraindicated.

SF was collected by aspiration, and the synovium was biopsied arthroscopically. Specimens were collected under general anesthesia at the time of AKO and plate removal. Plate removal was usually performed 1 to 2 years after surgery. The inclusion criterion was all of the patients who received AKO, according to the above surgical indication, and who received plate removal during the period. The exclusion criteria were as follows: Patients in whom either SF or synovium could not be collected at the time of AKO or plate removal, and with missing patient-reported outcomes (PROs).

Consent was based on verbal consent from individual subjects with an opt-out system. This study was approved by the Institutional Review Board of Kobe University (ID no. B190030)

Surgical Planning

Preoperative planning was performed using Miniaci's method,[30,31] based on the % weight-bearing line (%WBL). Target alignments were determined on the basis of lower limb alignment and OA severity assessed using preoperative radiographs. For knees with relatively mild OA (%WBL 20–40%), the target alignment was set at 55–60%. For advanced OA knees (%WBL <20%), a target alignment of 58–63% was set, while maintaining the postoperative medial proximal tibial angle within 95°.[32] If correction could not be achieved by opening-wedge HTO (OWHTO) alone, DLO was planned. In the presence of a patellofemoral joint, distal tibial tuberosity osteotomy (DTO) was planned.[33]

Surgical Procedures and Postoperative Management

For OWHTO, the medial proximal tibia was exposed using a straight incision, and the superficial fibers of medial collateral ligament was released distally. Biplane ascending and transverse cut was performed using oscillating bone saw and chisels. The osteotomy site was opened using an opener (Olympus Terumo

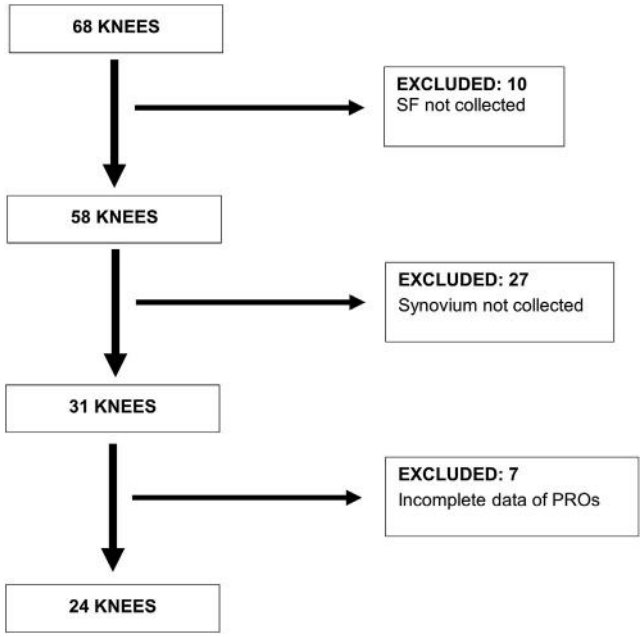

Fig 1. Flowchart showing the inclusion/exclusion process. SF, synovial fluid; PROs, patient-reported outcomes.

biomaterials Corp., Tokyo, Japan) or a spreader until intended alignment had been reached. The gap distance between the most posteromedial cortex was measured using a caliper. Two wedge-shaped, β-tricalcium phosphate blocks (OSferion 60, Olympus Terumo biomaterials Corp., Tokyo, Japan), depending on the size of the gap, were placed in the gap. A medial locking plate (TriS Medial HTO Plate System, Olympus Terumo Biomaterials Corp., Tokyo, Japan) was used to fix the tibia.

DTO was performed by cutting the tuberosity distally in the sagittal plane toward the anterior tibial cortex, instead of the ascending cut performed in HTO.[34]

Table 1. Patient Demographics and Baseline Characteristics[a]

Characteristics	Values
Age at time of osteotomy (y)	60.5 ± 1.2 (range: 47–68)
Sex (male/female)	10/14
Body mass index (kg/m^2)	26.8 ± 0.7 (range: 18.9–36.3)
Kellgren-Lawrence grade (1/2/3/4)	0/0/11/13
Surgical procedure (OWHTO/DTO/DLO)	11/8/5
Concomitant procedure	
Bone marrow stimulation (n)	12
Mosaic plasty (n)	1
Meniscectomy (n)	4
Meniscal repair (n)	7
Period from AKO to plate removal (months)	16.8 ± 1.5 (range: 11–38)

[a]AKO, around-knee osteotomy; DLO, double level osteotomy; DTO, distal tuberosity osteotomy; OWHTO, opening wedge high tibial osteotomy.

Table 2. Radiographic Changes and Patient-Reported Outcomes Before and After Surgery [a]

Assessment	Preoperative	Postoperative	*P* Value[b]
%WBL (%)	13.5 ± 3.7	57.5 ± 1.6	<.0001
KSS Activity Score	55.5 ± 3.5	86.7 ± 1.9	<.0001
KOOS			
Pain Score	48.0 ± 4.1	80.7 ± 3.2	<.0001
Symptom Score	48.1 ± 5.3	77.0 ± 3.6	<.0001
ADL Score	58.7 ± 5.6	87.8 ± 2.2	<.0001
Sports Score	28.8 ± 3.8	59.6 ± 4.2	<.0001
QOL Score	29.5 ± 3.9	59.8 ± 5.0	<.0001
IKDC Subjective Score	32.4 ± 1.7	65.0 ± 2.6	<.0001

[a]ADL, activity of daily living; IKDC, the International Knee Documentation Committee; KOOS, the knee injury and osteoarthritis outcome score; KSS, the Knee Society score; QOL, quality of life; WBL, weight-bearing line.
[b]Statistical significance: $P < .05$.

Cutting line was determined using an arc-osteotomy guide. Osteotomy site was opened and fixed according to OWHTO, with an additional 6.5-mm cannulated cancellous screw with a washer (Hollyx Co., Ltd., Shizuoka, Japan) inserted anteroposteriorly from the tibial tuberosity toward the posterior cortex.

When performing the DLO, a distal femoral osteotomy was added prior to OWHTO. The lateral distal femur was exposed using a straight incision, and lateral closing wedge osteotomy was performed, according to preoperative planning. The osteotomy gap was closed after removal of bone wedge, and the osteotomy side was fixed with a lateral locking plate (TriS DFO Plate System, Olympus Terumo Biomaterials Corp., Tokyo, Japan).

Partial weight-bearing was initiated one week after the surgery, and full weight-bearing was permitted 4 weeks after OWHTO and DTO, and 6 weeks after DLO. Once bony fusion was confirmed, plate removal was routinely performed ~1 to 2 years after surgery.

Patient-Reported Outcomes

Activity Score of the Knee Society Score (KSS),[35] Knee injury and Osteoarthritis Outcome Score (KOOS),[36] and International Knee Documentation Committee (IKDC) subjective score[37] were assessed as PROs before osteotomy and plate removal.

SF Sampling and Biomarker Assays

SF was collected by aspiration from the suprapatellar pouch before arthroscopic examination under general anesthesia. The total volume of SF samples was measured, and the samples were centrifuged for 15 min at 1,000 g within 30 min of collection. The supernatant was aliquoted and stored at −80°C until analysis. SF analysis was performed using a multiplex enzyme-linked immunosorbent assay kit: Human Luminex® Discovery Assay (F-RD-LuminexHM-03; R&D Systems,

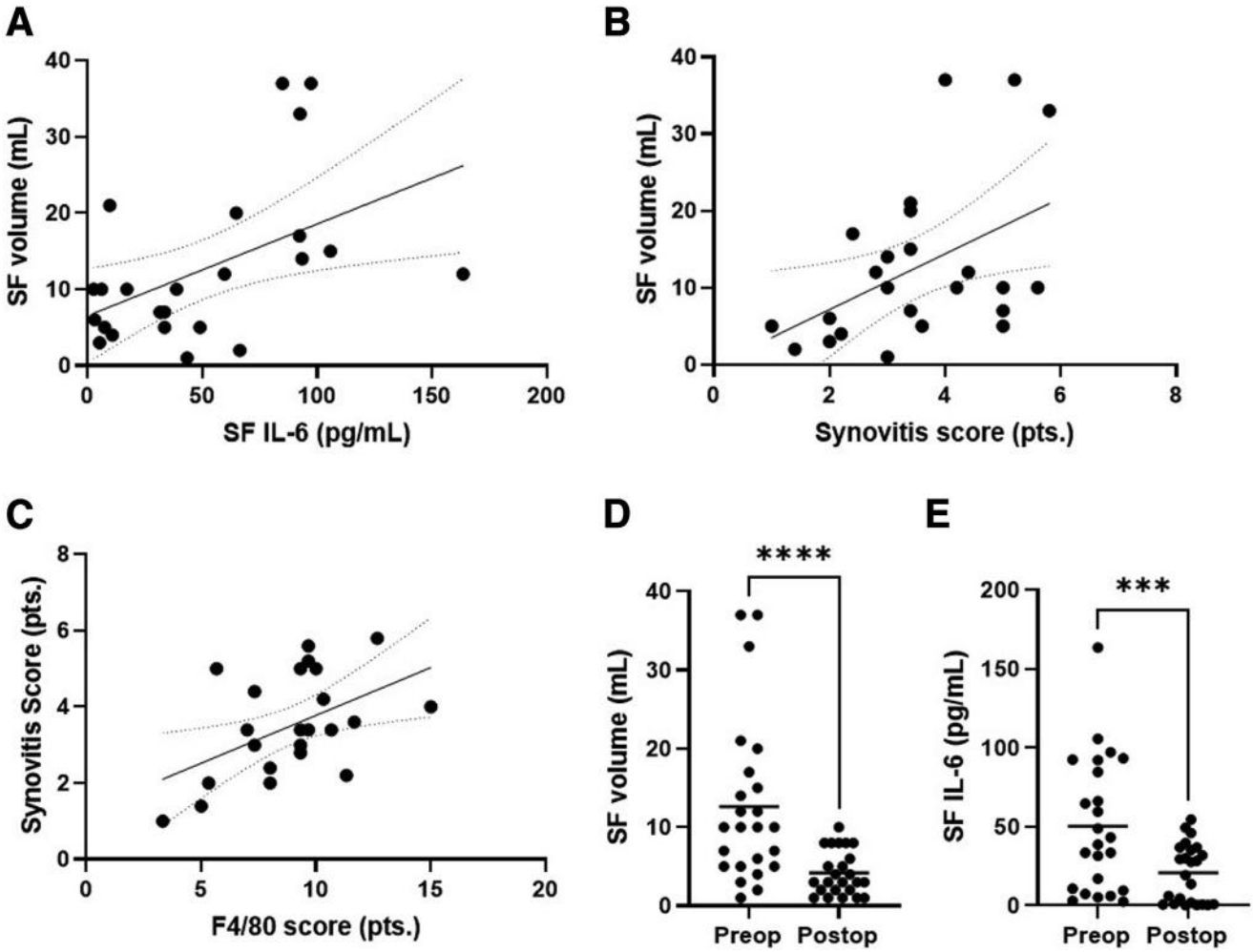

Fig 2. Preoperative correlations between IL-6 concentration in SF and SF volume (A), synovitis score and SF volume (B), and F4/80 score and synovitis score (C). Significant positive correlations were found in all the analyses. Preoperative and postoperative changes in SF volume (D) and IL-6 concentration (E) in SF. The total volume of SF was significantly decreased after surgery. The concentration of IL-6 in SF was significantly decreased after surgery. The lines in the scatterplot present the mean value. ***$P < .001$; ****$P < .0001$. IL, interleukin; Preop, preoperative; Postop, postoperative; SF, synovial fluid.

Minneapolis, MN) for IL-6 at a 1:2 dilution. Triplicate measurements were performed for all samples, and the average was calculated.

Synovium Sampling and Preparation

Arthroscopic exploration was performed before the osteotomy and plate removal. The intercondylar area, medial and lateral compartments, patellofemoral joint, and suprapatellar pouch were examined, and synovium specimens were taken from at least 2 sites of the most grossly inflamed area in the anteromedial compartment, including the infrapatellar fat pad. Biopsies were obtained from a similar area at the time of plate removal. The samples were promptly formalin-fixed for paraffin embedding and sectioned at a thickness of 6 μm. The sections were photographed using an all-in-one fluorescence microscope (BZ-X700; Keyence, Osaka, Japan), and cell counts were performed using the open-resource, digital image analysis software ImageJ (http://imagej.nih.gov/ij/).

Histopathology

Synovitis was evaluated semiquantitatively with hematoxylin & eosin (H&E) staining under 100× magnification, using a previously reported method by Lewis et al.[38] : a total of 0−6 points for enlargement of the synovial lining cell layer and density of the resident cells (0−3 points each). The evaluation was performed by an orthopaedic surgeon (SW) trained by a laboratory technician using five randomly selected sections, and the mean was calculated. To identify bone and cartilage fragments on the synovium, van Gieson staining was performed and assessed under a 400× high-power field (HPF).[11]

Table 3. Changes in SF and Synovium Before and After Surgery[a]

Assessment	Preop.[b]	Postop.[b]	*P* Value[c]
SF volume (mL)	12.6 ± 2.1	4.2 ± 0.6	<.0001
SF IL-6 concentration (pg/mL)	50.5 ± 8.6	20.7 ± 3.8	.0001
Synovitis score (pts.)	3.5 ± 0.3	1.8 ± 1.0	.0001
F4/80 score (pts.)	8.9 ± 0.5	6.6 ± 0.4	.0003
M1/M2 ratio	1.7 ± 0.1	1.2 ± 0.1	.0007
IL-1β-positive cells (%)			
Entire synovium	43.5 ± 2.4	37.3 ± 2.4	.13
Intimal layer	53.8 ± 2.8	42.9 ± 2.4	.008
IL-6-positive cells (%)			
Entire synovium	34.0 ± 2.3	32.3 ± 1.7	.55
Intimal layer	49.7 ± 3.0	38.6 ± 1.7	.002
Blood vessels per HPF	7.7 ± 0.7	4.3 ± 0.3	<.0001

[a]HPF, high-power field; IL, interleukin; Postop, postoperative; Preop, preoperative; SF, synovial fluid; pts, points.
[b]Data are expressed as means ± SE.
[c]Statistical significance: $P < .05$.

Immunohistochemistry

Before staining, the slides were preincubated for 15 min at 37.0°C. After deparaffinization with xylene, the slides were hydrated using a graded ethanol series and washed with distilled water. For antigen retrieval, slides were digested with proteinase K (ready-to-use; Dako, Glostrup, Denmark) at room temperature for 10 min. After rinsing in phosphate-buffed saline (PBS) (Dulbecco's PBS [−]; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) for 5 min, the slides were incubated in 3% H_2O_2/methanol for 30 min to quench endogenous peroxidase activity. After further rinsing in PBS for 5 minutes, sections were applied with primary antibodies diluted in Can Get Signal Immunoreaction Enhancer Solution (Toyobo Co., Ltd., Osaka, Japan): rabbit polyclonal F4/80 antibody (27044-1-AP; Proteintech Group, Chicago, IL; 1:100 dilution); rabbit polyclonal CD80 antibody (bs-2211R; Bioss, Inc., Boston, MA; 1:200 dilution); rabbit polyclonal CD163 antibody (bs-2527R; Bioss, Inc.; 1:200 dilution); rabbit polyclonal IL-1β antibody (16806-1-AP; Proteintech Group; 1:100 dilution); rabbit polyclonal IL-6 antibody (21865-1-AP; Proteintech Group; 1:100 dilution); rabbit polyclonal CX_3CR1 antibody (13885-1-AP; Proteintech Group; 1:100 dilution); and rabbit polyclonal vascular endothelial growth factor (VEGF) antibody (19003-1-AP; Proteintech Group; 1:100 dilution). A negative control was prepared using PBS. The sections were incubated overnight at 4°C, washed three times with PBS, and then incubated with a secondary anti-rabbit IgG antibody (Histofine Simple Stain MAX PO; Nichirei Bioscience, Tokyo, Japan) for 1 h at room

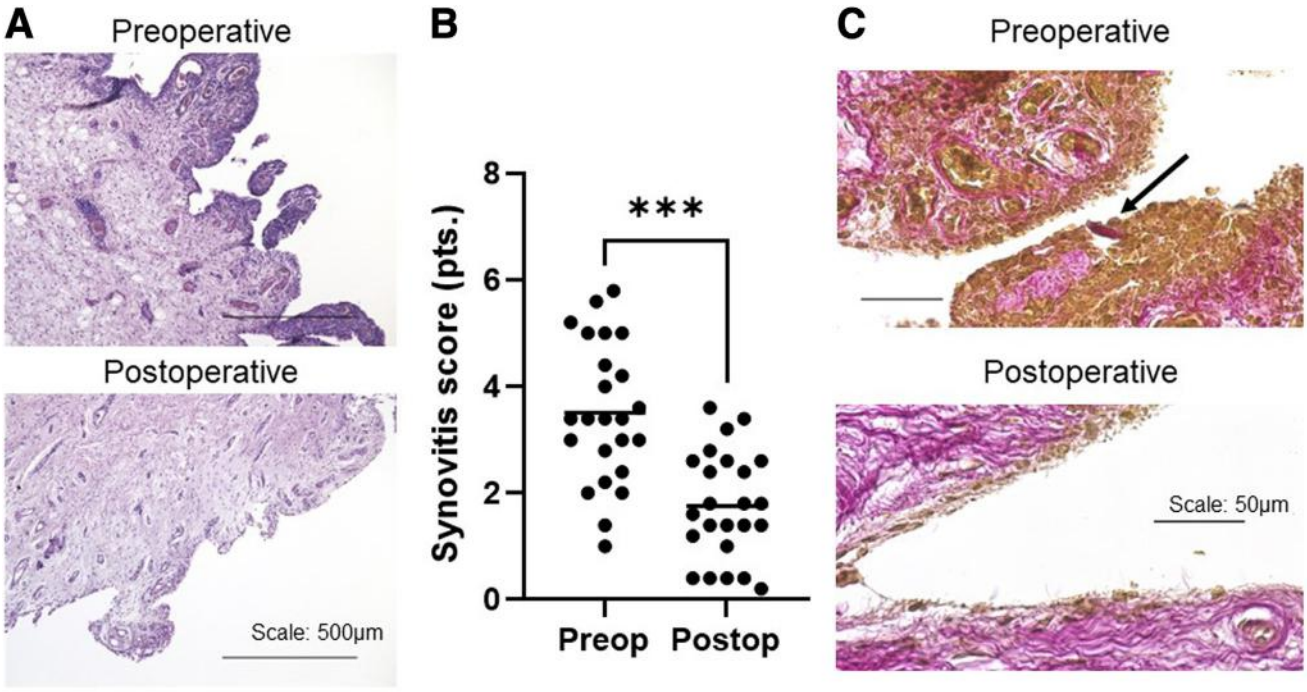

Fig 3. Histological findings in the synovium. (A) Typical preoperative and postoperative images of H&E staining of the synovial membrane. (B) Changes in the synovitis score. (C) Preoperative and postoperative images of van Gieson staining. Synovitis score was significantly improved after AKO. The lines in the scatterplot present the mean value. The black arrow indicates osteochondral fragments stained pink/red with van Gieson staining in the intimal layer. ***$P < .001$. AKO, around-knee osteotomy; H&E, hematoxylin and eosin; Postop, postoperative; Preop, preoperative.

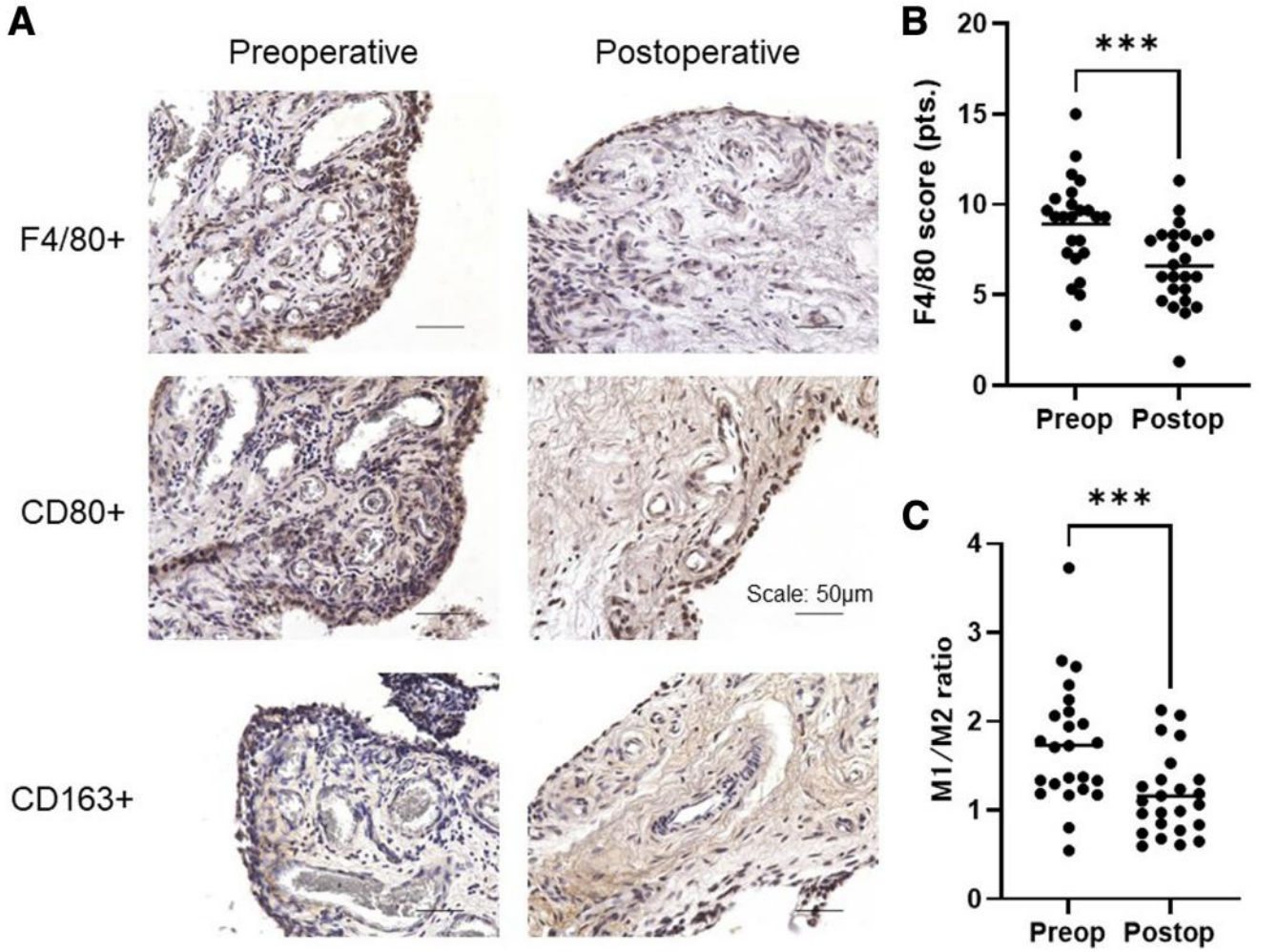

Fig 4. Immunohistochemistry for macrophage markers. (A) Typical images of F4/80, CD80, and CD163-positive cell infiltration before and after AKO. (B) Comparison of F4/80 score before and after surgery. (C) Comparison of M1/M2 ratio before and after surgery. F4/80 score and M1/M2 ratio were significantly decreased after surgery. The lines in the scatterplot present the mean value. ***$P < .001$. AKO, around-knee osteotomy; Preop, preoperative; Postop, postoperative.

temperature. After 3 washes with PBS, the sections were incubated with peroxidase substrate 3,3′-diaminobenzidine (Histofine Simple Stain DAB solution; Nichirei Bioscience, Tokyo, Japan) for 5 min. Hematoxylin was used as the counterstain. Finally, the slides were washed with distilled water, dehydrated with a graded alcohol series, permeabilized with xylene, sealed, and examined under an optical microscope.

F4/80 expression, which is a pan-macrophage marker, was evaluated as an immunoinflammatory cell marker using a previously reported scoring system.[39] The scores were assessed at 2 randomly selected sites on the synovium for the superficial and deep layers of the intima and were calculated as the sum of these 4 sites (16-point scale). The percentage of cells positive for CD80 and CD163 were assessed as M1 and M2 macrophage markers, respectively, to calculate the M1/M2 ratio. The characterization of intimal macrophages was qualitatively assessed by the expression of CX_3CR1. The expression of proinflammatory cytokines in cells was assessed by the percentage of IL-1β- and IL-6-positive cells in the entire synovium and intima only, respectively. The number of subintimal blood vessels was calculated as the number of VEGF-positive luminal structures. All assessments were performed by SW at 400× HPF at three randomly selected locations on three randomly selected sections, and the mean values were calculated.

Statistical Analysis

All analyses were performed using GraphPad Prism version 9.4.1 for Windows (GraphPad Software, Inc., San Diego, CA). The D'Agostino-Pearson normality test was used to assess the normal distribution of each parameter. Preoperative and postoperative changes in PROs were assessed using Student's *t*-test. Correlations between the preoperative IL-6 concentration and SF volume, synovitis score, SF volume, F4/80 score, and synovitis score were assessed using simple linear

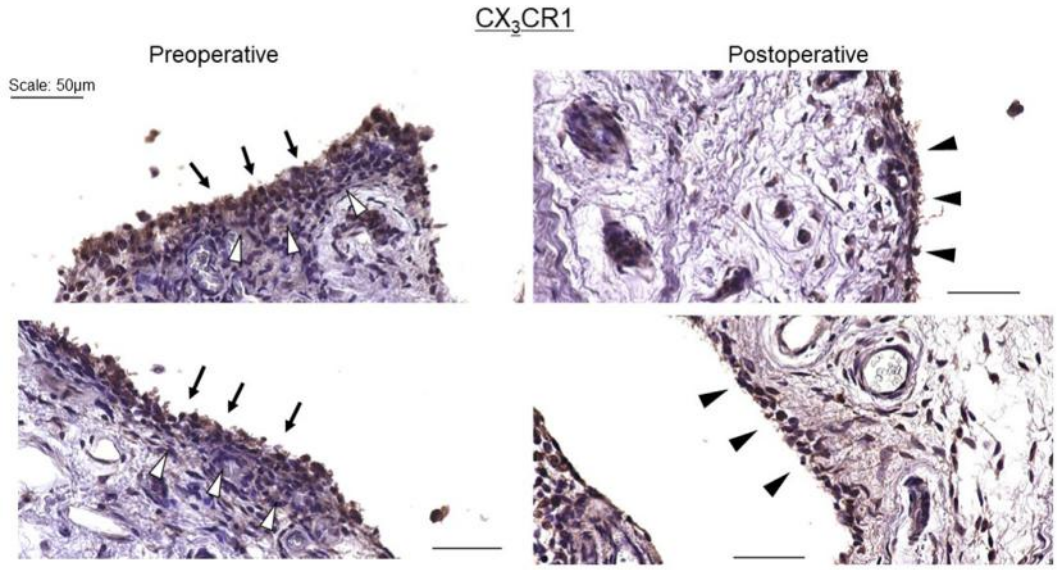

Fig 5. Immunohistochemistry for CX_3CR1. Preoperatively, CX3CR1-positive macrophages are arranged in the superficial layers of the intima, with variation in morphology and orientation (black arrows). There is also infiltration of CX_3CR1-negative macrophages in the deeper layers (white arrowheads). Postoperatively, CX_3CR1-positive macrophages maintain their position in the superficial layers of the intima and are stable in morphology and arrangement (black arrowheads). The infiltration of CX_3CR1-negative macrophages in the deeper layers was decreased.

regression analysis. Wilcoxon matched-pairs signed rank test was used to test for differences in within-subject changes before and after osteotomy in SF volume, IL-6 concentration in SF, synovitis score, F4/80 score, M1/M2 ratio, percentage of IL-1β and IL-6 positive cells, and number of blood vessels. Statistical significance was set at $P < .05$. Post hoc power analysis of Wilcoxon matched-pairs, signed-rank test was performed using G*Power for SF volume and IL-6 concentration. The power analysis for synovial fluid volume and IL-6 concentration showed actual power of 0.98 and 0.93, respectively.

Reliability of the synovitis score, F4/80 score, and number of blood vessels were assessed by two orthopaedic surgeons (SW and KK) who were blinded to the clinical information, using a three-time evaluation of 10 randomly selected sections. The Interclass Correlation Coefficient (ICC) for intra- and inter-rater reliabilities were analyzed using EZR version 1.60 (Saitama Medical Center, Jichi Medical University, Saitama, Japan). For the synovitis score, the intra-rater reliability of reader 1 was 0.90 (95% confidence interval [CI] 0.74, 0.97) for single measures and 0.96 (95% CI 0.90, 0.99) for single measures and of reader 2 was 0.84 (95% CI 0.63, 0.95) for single measures and 0.94 (95% CI 0.84, 0.98) for average measures. ICC values for inter-rater reliability of the synovitis score were 0.94 (95% CI 0.77, 0.98) for single measures and 0.99 (95% CI 0.97, 1.00) for average measures. For the F4/80 score, the intra-rater reliability of reader 1 was 0.82 (95% CI 0.58, 0.95) for single measures and 0.93 (95% CI 0.80, 0.98) for average measures and that of reader 2 was 0.77 (95% CI 0.48, 0.93) for single measures and 0.91 (95% CI 0.74, 0.98) for average measures. ICC values for inter-rater reliability of the F4/80 score were 0.89 (95% CI 0.54, 0.97) for single measures and 0.93 (95% CI 0.73, 0.98) for average measures. For the number of blood vessels, the intra-rater reliability of reader 1 was 0.94 (95% CI 0.84, 0.98) for single measures and 0.98 (95% CI 0.94, 0.99) for single measures and of reader 2 was 0.87 (95% CI 0.69, 0.96) for single measures and 0.95 (95% CI 0.87, 0.99) for average measures. ICC values for inter-rater reliability of the number of blood vessels were 0.90 (95% CI 0.59, 0.97) for single measures and 0.97 (95% CI 0.81, 0.99) for average measures. All of the evaluation methods showed good to excellent agreement.[40]

Results

Patient Demographics and Clinical outcomes

A total of 24 knees (10 males/14 females; mean age: 60.5 ± 1.2 years) of 21 patients were included (Fig 1). Patient demographics are shown in Table 1. All subjects had Kellgren-Lawrence grade 3 or 4 knee OA.[41] At plate removal with a mean time of 16.8 months (range: 11−38 months) postoperatively, %WBL was significantly altered from 13.5% ± 3.7% to 57.5% ± 1.6%. The clinical outcomes, as assessed by the KSS, IKDC, and KOOS, were significantly improved after AKO (Table 2). Minimal clinically important difference[42] was achieved as follows: KSS Activity Score, 21 knees

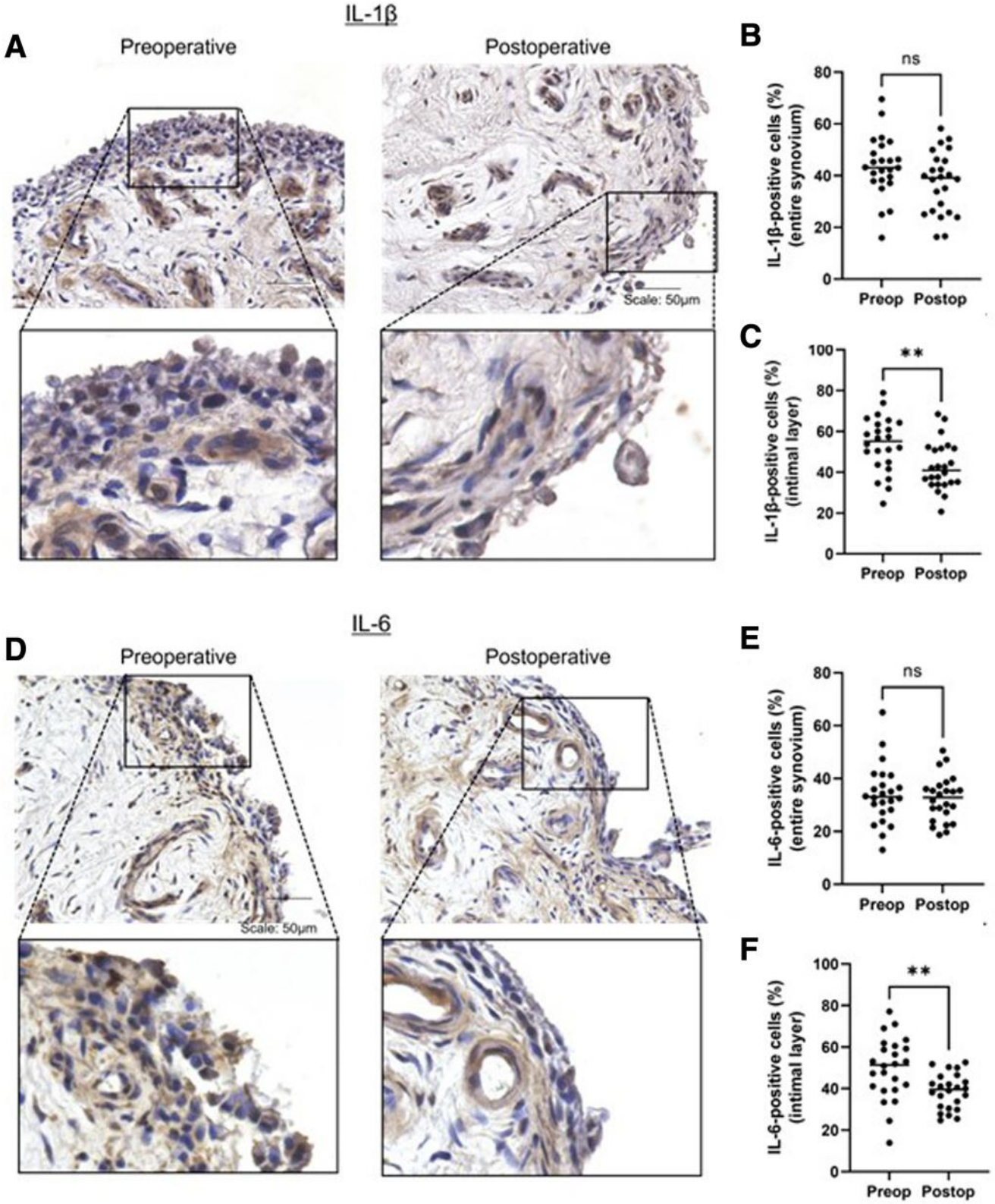

Fig 6. Immunohistochemistry for proinflammatory cytokines. Comparison of IL-1β-positive (A) and IL-6-positive cells (D) before and after AKO. The area boxed in the top row is enlarged and displayed in the bottom row. Comparison of IL-1β-positive (B) and IL-6-positive (E) cells (%) before and after surgery in the entire synovium. Comparison of IL-β-positive (C) and IL-6-positive (F) cells (%) before and after surgery in the initial layer. The lines in the scatterplot present the mean value. There was no statistically significant difference in IL-1β- and IL-6-positive cells (%) in the entire synovium, whereas they were significantly reduced in the intima. $^{**}P < .01$. ns, not significant; IL, interleukin; Preop, preoperative; Postop, postoperative.

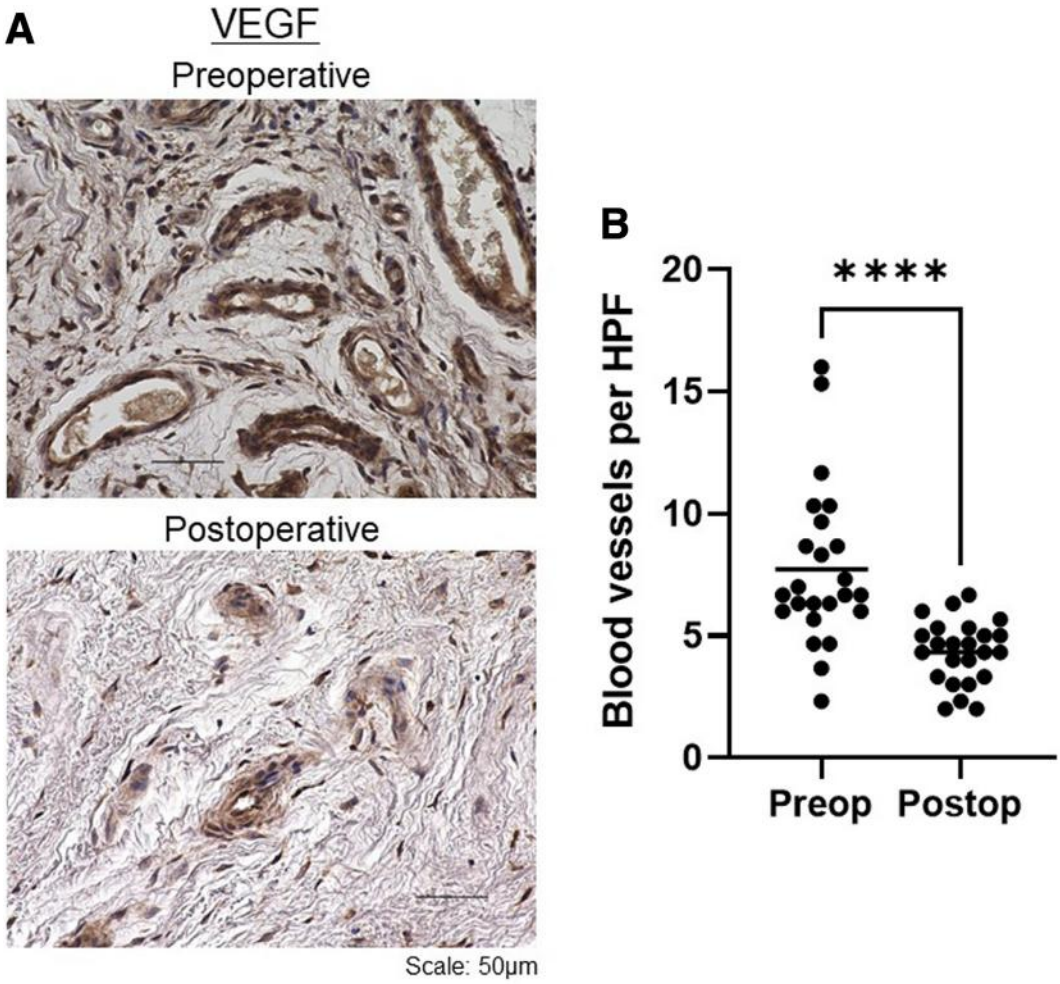

Fig 7. Immunohistochemistry for VEGF. The number of blood vessels was determined from the VEGF-positive luminal structures. (A) Typical preoperative and postoperative images. (B) Comparison of the number of blood vessels before and after AKO. The lines in the scatterplot present the mean value. Vessel counts significantly decreased after AKO. $^{**}P < .01$. AKO, around-knee osteotomy; HPF, high-power field; Postop, postoperative; Preop, preoperative; VEGF, vascular endothelial growth factor.

(87.5%), KOOS Pain, 17 knees (70.8%), Symptom, 18 knees (75%), ADL, 15 knees (62.5%), Sports, 18 knees (75%), and QOL, 19 knees (79.2%).

Analysis of Preoperative Synovial Fluids

Preoperatively, SF volume was significantly correlated with IL-6 concentration in the SF ($P = .02$, $R = 0.49$) and synovitis score ($P = .02$, $R = 0.46$), and a positive correlation was also found between synovitis score and F4/80 score ($P = .02$, $R = 0.49$) (Fig 2, A–C).

Synovial Fluid Volume and Interlukin-6 Concentration in the Synovial Fluid

At the time of plate removal, SF volume significantly decreased (12.6 ± 2.1 mL vs 4.2 ± 0.6 mL; $P < .0001$) (Fig 2D, Table 3), and the concentrations of IL-6 in SF were also significantly decreased compared with those preoperatively (50.5 ± 8.6 pg/mL vs 20.7 ± 3.8 pg/mL; $P = .0001$) (Fig 2E, Table 3).

Histopathological Analysis of the Synovium

A photograph of H&E staining of the synovium is shown in Fig 3A. The synovial lining cell layer and infiltrating cells in the subintima decreased, and there was less vascularity after AKO. Semiquantitative scoring for synovitis showed significant improvement after AKO (3.5 ± 0.3 points vs 1.8 ± 1.0 points, $P = .0001$) (Fig 3B, Table 3). Van Gieson staining showed pink cartilaginous debris in all preoperative synovial specimens. In contrast, similar fragments were identified in only 25% of synovial specimens postoperatively (Fig 3C).

Analysis of Macrophage Makers

The F4/80 score, which represents macrophage infiltration, significantly decreased after AKO (8.9 ± 0.5 vs 6.6 ± 0.4 points; $P = .0003$) (Fig 4, A and B, Table 3). The mean CD80-positive cell rate was 53.6% ± 2.8% preoperatively and 40.3% ± 3.3% postoperatively, and

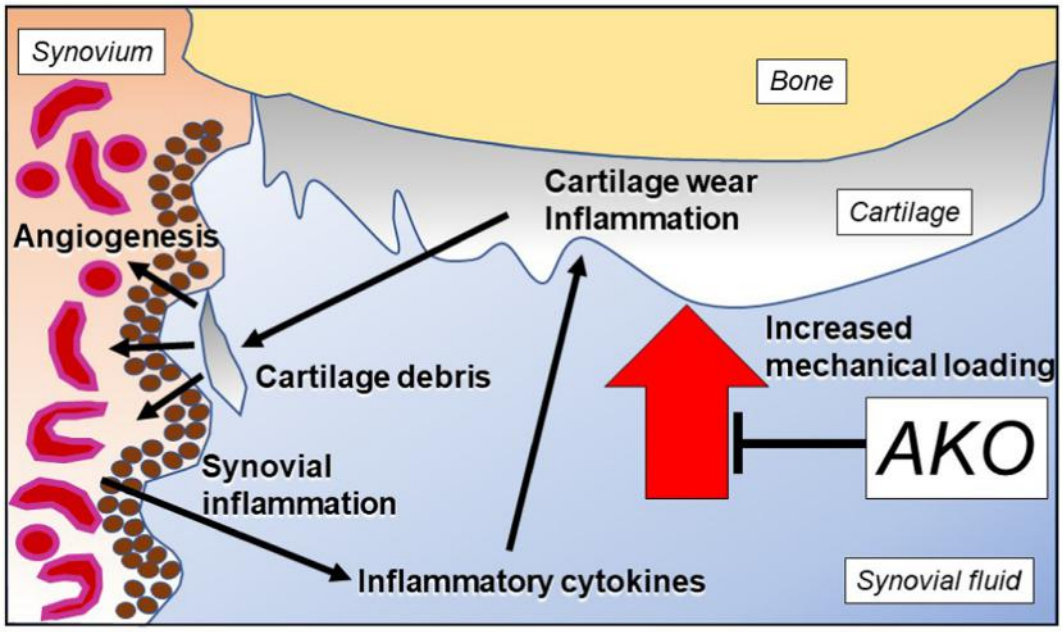

Fig 8. Our proposed mechanism illustrates the effect of AKO on joint environment. Increased mechanical loading due to abnormal limb mechanical alignment results in cartilage wear and inflammation. Cartilage debris from microcartilage wear is captured by the synovium, triggering synovial inflammation and angiogenesis. The inflamed synovium releases inflammatory cytokines into the synovial fluid, causing further cartilage wear and inflammation. AKO alters lower limb alignment, thereby reducing the increased joint loading and may have an inhibitory effect on a series of cascades. AKO, around-knee osteotomy.

the mean CD163-positive cell rate was 34.3% ± 2.5% preoperatively and 37.7% ± 3.6% postoperatively. The M1/M2 ratio calculated from these data decreased significantly postoperatively (1.7 ± 0.1 vs 1.2 ± 0.1; $P = .0007$) (Fig 4, A and C, Table 3). CX_3CR1-positive cells were observed in the superficial layer of the intima both preoperatively and postoperatively. However, the preoperative arrangement of CX_3CR1-positive cells was disorganized, and infiltration of multilayered CX_3CR1-negative cells was observed in the underlying layers (Fig 5).

The percentage of IL-1β-positive cells did not significantly change postoperatively when assessed in the entire synovium (43.5 ± 2.4% vs 37.3 ± 2.4; $P = .13$) (Fig 6, A and B, Table 3); however, it decreased significantly when assessed specifically in the intima (53.8 ± 2.8 vs 42.9 ± 2.4%; $P = .008$) (Fig 6, A and C, Table 3). The percentage of IL-6-positive cells was similar across the entire synovium (34.0 ± 2.3% vs 32.3 ± 1.7%; $P = .55$) (Fig 6, D and E, Table 3) and intima (49.7 ± 3.0% vs 38.6 ± 1.7%; $P = .002$) (Fig 6, D and F, Table 3). The number of VEGF-positive vasculatures per HPF in the subintimal layer significantly decreased after AKO (7.7 ± 0.7 vs 4.3 ± 0.3; $P < .0001$) (Table 3 and Fig 7).

Discussion

The main finding of this study was that the SF volume and IL-6 concentration decreased after AKO. In addition, synovitis decreased in association with reduced synovial angiogenesis in the subintima, inflammatory cytokine-positive cells in the intima, inflammatory status of macrophages, and improved layer structure of the synovium after AKO. These results suggest that the biological environment within the knee joint could improve with improved biomechanical conditions after AKO (Fig 8).

A previous study reported that an increase in SF volume over 1 year have been reported to be associated with cartilage loss, OA progression, and risk of total knee arthroplasty.[43] In addition, SF volume assessed by MRI was correlated with semiquantitative synovitis scores in patients with RA and OA.[13] We also found a positive correlation between the preoperative synovitis score and SF volume. Furthermore, a positive correlation between IL-6 concentration in SF and SF volume was observed in preoperative samples. These reports suggest that SF volume is an indicator of OA progression and is closely associated with synovial inflammatory activity. In the present study, SF volume and IL-6 concentration in SF and histological synovitis significantly decreased after AKO compared with the preoperative status, suggesting that AKO could improve synovial inflammation associated with SF volume and inflammatory cytokines. The period from AKO to plate removal was inconsistent; however, it was 1−2 years in most cases, and no correlation was found between any parameters and the period from AKO to plate removal. Notably, cartilaginous debris was found in all

Table 4. Comparison Between the Present Study and Previous Studies in Terms of IL-6 Concentration in SF[a]

Lead Author Year	Subjects	Mean Age (Years)	Method	SF IL-6 (pg/mL)[b]
Rübenhagen, R[47] 2012	Knees underwent surgery (KL grade 0–4)	29	ELISA	53#
Sohn, DH[48] 2012	OA (KL grade 2–4)	65.4	Bead-based immunoassay	975.4#
Beekhuizen, M[14] 2013	Healthy knees (donors within 24 hours after death)	39.6	ELISA	4.8#
	End-stage OA	69.9	ELISA	135.8#
Kusayama, Y[15] 2014	OA (KL grade 2–3) (pre-HA injection)	66.7	CLEIA	3458*
	OA (post HA injection)	Not reported	CLEIA	486*
Kaplan, DJ[49] 2017	Healthy knees (contralateral side)	41.1	Bead-based immunoassay	21.0*
	ACL tear with cartilage damage	36.3	Bead-based immunoassay	400*
	ACL tear without cartilage intact	34.0	Bead-based immunoassay	191*
Li, L[16] 2020	OA (KL grade 1–4)	63.4	ELISA	29.3*
Matejova, JP[46] 2020	OA female	Not reported	ELISA	32.55#
	OA male	Not reported	ELISA	55.63#
	OA (KL grade ≤2)	56.8	ELISA	55.63#
	OA (KL grade ≥3)	69.9	ELISA	32.73#
Watt, FE[17] 2020	OA (KL grade ≥2)	55	ECL	11.3#
Kumagai, K[18] 2021	OA (pre-HTO)	66.1	ELISA	745.6*
	OA (post-HTO)	Not reported	ELISA	300.2*
The present study	OA (pre-AKO)	60.5	ELISA	50.5*
	OA (post-AKO)	61.9	ELISA	20.7*

[a]ACL, anterior cruciate ligament; AKO, around-knee osteotomy; CLEIA, chemiluminescent enzyme immunoassay; ECL, electrochemiluminescence; ELISA, enzyme-linked immunosorbent assays; HA, hyaluronic acid; HTO, high tibial osteotomy; KL, Kellgren–Lawrence; OA, osteoarthritis.

[b]Data are expressed as *mean or #median.

preoperative samples, but only in 25% of the samples at the time of plate removal. The capture of cartilage fragments from worn joint surfaces in the inflamed synovium has been observed previously,[8,11,12] and synovitis in knee OA has been reported to occur in the synovium located in the vicinity of the degenerated cartilage.[44,45] These observations suggest that reduced synovial inflammation is a consequence of decreased cartilage wear after AKO.

Previous studies have reported that elevated IL-6 concentration in SF from the knee joints of patients with OA[14–18,46–48] and IL-6 in SF may influence the pathogenesis of OA or represent a response to the condition. There are several reports on changes in IL-6 during the course of treatment for OA. Kumagai et al. reported a decrease in IL-6 levels after HTO;[18] Kusayama et al. reported a decrease in IL-6 after intra-articular injection of hyaluronic acid.[15] In the present study, similar results were observed after AKO, whereas the levels of IL-6 concentrations in SF are highly variable in previous reports (Table 4); the SF IL-6 concentrations in OA knees ranged from 29.3 to 745.6 pg/mL.[14–18,47–49] Although the exact reason is unknown, the variability may be due to differences in methodology or patient background. Regarding "normal" IL-6 concentration in SF, Beekhuizen et al.[14] reported that the median IL-6 concentration in SF from healthy donors was 4.6 pg/mL, whereas Kaplan et al.[49] reported that the mean IL-6 concentration was 21.0 pg/mL in the SF from contralateral knees of patients with anterior cruciate ligament injury. Although the difference between the two studies was significant, these values may be considered as reference values. In the present study, the mean preoperative SF IL-6 concentration was 50.5 ± 8.6 pg/mL. Although the values widely ranged, it was within the range of concentration in previous reports of OA knees. Meanwhile, the mean IL-6 concentration was 20.7 ± 3.8 pg/mL at the time of plate removal, which was similar or possibly higher than normal concentration. These observations suggest that joint inflammation can be reduced after AKO, but there may be some residual inflammation.

Synovial angiogenesis has been suggested as a characteristic finding of OA synovitis.[50,51] In the present study, synovitis was reduced after AKO, in association with reduced synovial angiogenesis in the subintimal layer, as assessed by the number of VEGF-positive luminal structures. Although the mechanisms

underlying the reduction in angiogenesis are currently unknown, it appears to be a secondary consequence of reduced inflammation. Meanwhile, the ratio of IL-1β- and IL-6-positive cells in the entire synovium did not significantly change before and after AKO. However, the ratios of IL-1β- and IL-6-positive cells were significantly reduced postoperatively when the intimal layer was assessed separately. As multilayering of the intima is the most typical finding in OA synovium,[4,11] changes in the intima may sensitively reflect the joint condition and synovitis. Since inflammatory cytokines such as IL-1β and tumor necrosis factor-α induce the expression of collagenase and aggrecanase in cartilage;[52,53] thereby, the reduced inflammatory cytokine expression in the synovial intima may lead to reduction in cartilage catabolic responses. Further study is required to elucidate the interaction between cartilage and synovium.

Studies have shown that the polarity of synovial macrophages is closely associated with the development of OA. Zhang et al.[10] found that in a mouse model of OA, macrophages in the synovium and joint space aggregated, increasing M1 synovial macrophages and promoting OA progression. It has also been shown that M1 macrophages increase and M2 macrophages decrease in the synovium during OA development.[9] In the present study, synovitis score was improved in association with decreased macrophage infiltration, and M1/M2 polarity shifted from M1-dominant state toward M2 after surgery, suggesting that macrophage status played a role in the improved synovitis. Recently, it has been reported that a distinct subset of the macrophage population residing in the synovium plays different roles in joint homeostasis and inflammation. Membrane-forming macrophages selectively express CX_3CR1 and form a dense barrier between the synovium and joint cavity, whereas CX_3CR1-negative macrophages proliferate in association with disruption of the barrier in responded to inflammation.[54] In the present study, a disorganized arrangement of CX_3CR1-positve synovial lining cells was observed preoperatively, whereas CX_3CR1-positve synovial cells were well aligned in the superficial layer of the synovium in the samples obtained at the time of second-look arthroscopy. Therefore, these observations suggested that not only the macrophage phenotype but also the macrophage-forming microstructure of the synovium achieved a more physiological state and was associated with reduced inflammation.

Limitations

The present study has several limitations. First, patients who did not have sufficient postoperative SF were excluded. The results may be different if these cases were included. Second, the influence of synovectomy or abrasion chondroplasty performed at the time of AKO cannot be excluded. However, the data in this study may represent the biological changes after AKO in common clinical settings.

Conclusions

SF volume and IL-6 concentrations in the SF decreased, and inflammatory synovium pathology improved after AKO. In addition to biomechanical changes, the biological environment of the joint can be improved after AKO.

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