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

Abstract

Purpose: The purpose of this study was 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 pro-inflammatory 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 ($P < .008$, $P < .002$ 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.

Key words: Knee osteoarthritis, Synovitis, Synovial fluid, Macrophage, Angiogenesis, Inflammation, Around-knee osteotomy

Introduction

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, 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.

Materials and Methods

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 defect was 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 one to two years after surgery. The inclusion criterion was all the patients who received AKO according to the above surgical indication and 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 based on 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 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 (OSferion60, 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 towards the anterior tibial cortex, instead of ascending cut performed in HTO.[34] 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 antero-posteriorly from the tibial tuberosity towards the posterior cortex.

When performing DLO, 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 removed 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 four weeks after OWHTO and DTO, and six weeks after DLO. Once bony fusion was confirmed, plate removal was routinely performed approximately 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 1000 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, Minneapolis, MN, USA) 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 two 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 semi-quantitatively 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]

Immunohistochemistry

Before staining, the slides were preincubated for 15 min at 37.0℃. 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 rinse 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, USA; 1:100 dilution); rabbit polyclonal CD80 antibody (bs-2211R; Bioss Inc, Boston, MA, USA; 1:200 dilution); rabbit polyclonal CD163 antibody (bs-2527R; Bioss Inc, Boston, MA, USA; 1:200 dilution); rabbit polyclonal IL-1β antibody (16806-1-AP; Proteintech Group, Chicago, IL, USA; 1:100 dilution); rabbit polyclonal IL-6 antibody (21865-1-AP; Proteintech Group, Chicago, IL, USA; 1:100 dilution); rabbit polyclonal CX_3CR1 antibody (13885-1-AP; Proteintech Group, Chicago, IL, USA; 1:100 dilution); and rabbit polyclonal vascular endothelial growth factor (VEGF) antibody (19003-1-AP; Proteintech Group, Chicago, IL, USA; 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 [R]; Nichirei Bioscience, Tokyo, Japan) for 1 h at room temperature. After three 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 two randomly selected sites on the synovium for the superficial and deep layers of the intima and were calculated as the sum of these four 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 pro-inflammatory 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, USA). The D'Agostino-Pearson normality test was used to assess the normal distribution of each parameter. Pre- 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 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 the evaluation methods showed good–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 (**Figure 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 (87.5%); KOOS Pain, 17 knees (70.8%); Symptom, 18 knees (75%); ADL, 15 knees (62.5%); Sports, 18 knees (75%); QOL, 19 knees (79.2%).

227

228 **Analysis of preoperative synovial fluids**

229 Preoperatively, SF volume was significantly correlated with IL-6 concentration in the SF (P = .02, R =

230 0.49) and synovitis score (P = .02, R = 0.46), and a positive correlation was also found between

231 synovitis score and F4/80 score (P = .02, R = 0.49) (**Figures 2A–C**).

232

233 **Synovial fluid volume and interlukin-6 concentration in the synovial fluid**

234 At the time of plate removal, SF volume significantly decreased (12.6 ± 2.1 mL vs 4.2 ± 0.6 mL, P < .0001)

235 (**Figure 2D, Table 3**), and the concentrations of IL-6 in SF were also significantly decreased compared

236 with those preoperatively (50.5 ± 8.6 pg/mL vs 20.7 ± 3.8 pg/mL, P = .0001) (**Figure 2E, Table 3**).

237

238 **Histopathological analysis of the synovium**

239 A photograph of H&E staining of the synovium is shown in **Figure 3A**. The synovial lining cell layer and

240 infiltrating cells in the subintima decreased, and there was less vascularity after AKO. Semiquantitative

241 scoring for synovitis showed significant improvement after AKO (3.5 ± 0.3 pts. vs 1.8 ± 1.0 pts., P

242 = .0001) (**Figure 3B, Table 3**). Van Gieson staining showed pink cartilaginous debris in all preoperative

243 synovial specimens. In contrast, similar fragments were identified in only 25% of synovial specimens

244 postoperatively (**Figure 3C**).

245

246 **Analysis of macrophage makers**

247 The F4/80 score, which represents macrophage infiltration, significantly decreased after AKO (8.9 ± 0.5
248 pts. vs 6.6 ± 0.4 pts., P = .0003) (**Figure 4A, B, Table 3**). The mean CD80-positive cell rate was 53.6%
249 ± 2.8% preoperatively and 40.3% ± 3.3% postoperatively, and the mean CD163-positive cell rate was
250 34.3% ± 2.5% preoperatively and 37.7% ± 3.6% postoperatively. The M1/M2 ratio calculated from these
251 data decreased significantly postoperatively (1.7 ± 0.1 vs 1.2 ± 0.1, P = .0007) (**Figure 4A, C, Table 3**).
252 CX_3CR1-positive cells were observed in the superficial layer of the intima both pre- and post-operatively.
253 However, the preoperative arrangement of CX_3CR1-positive cells was disorganized, and infiltration of
254 multi-layered CX_3CR1-negative cells was observed in the underlying layers (**Figure 5**).

255 The percentage of IL-1β-positive cells did not significantly change postoperatively when assessed in the
256 entire synovium (43.5 ± 2.4% vs 37.3 ± 2.4, P = .13) (**Figure 6A, B, Table 3**); however, it decreased
257 significantly when assessed specifically in the intima (53.8 ± 2.8 vs 42.9 ± 2.4%, P = .008) (**Figure 6A,**
258 **6C, Table 3**). The percentage of IL-6-positive cells was similar across the entire synovium (34.0 ± 2.3%
259 vs 32.3 ± 1.7%, P = .55) (**Figure 6D, E, Table 3**) and intima (49.7 ± 3.0% vs 38.6 ± 1.7%, P = .002)
260 (**Figure 6D, 6F, Table 3**). The number of VEGF-positive vasculatures per HPF in the subintimal layer
261 significantly decreased after AKO (7.7 ± 0.7 vs 4.3 ± 0.3, P < .0001) (**Table 3**) **(Figure 7)**.

262

263 ## 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 **(Figure 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 semi-quantitative 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, 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. Meanwhile, 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. Notably, cartilaginous debris was found in all preoperative samples, but only in 25% of the

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

285 Previous studies have reported that elevated IL-6 concentration in SF from the knee joints of patients with
286 OA[14–18,46–48] and IL-6 in SF may influence the pathogenesis of OA or represent a response to the condition.
287 There are several reports on changes in IL-6 during the course of treatment for OA. Kumagai et al. reported
288 a decrease in IL-6 levels after HTO;[18] Kusayama et al. reported a decrease in IL-6 after intra-articular
289 injection of hyaluronic acid.[15] In the present study, similar results were observed after AKO, whereas the
290 levels of IL-6 concentrations in SF are highly variable in previous reports (**Table 4**); the SF IL-6
291 concentrations in OA knees ranged from 29.3–745.6 pg/mL.[14–18,47–49] Although the exact reason is
292 unknown, the variability may be due to differences in methodology or patient background. Regarding
293 "normal" IL-6 concentration in SF, Beekhuizen et al.[14] reported that the median IL-6 concentration in SF
294 from healthy doners was 4.6 pg/mL, whereas Kaplan et al.[49] reported that the mean IL-6 concentration
295 was 21.0 pg/mL in the SF from contralateral knees of patients with anterior cruciate ligament injury.
296 Although the difference between the two studies was significant, these values may be considered as
297 reference values. In the present study, the mean preoperative SF IL-6 concentration was 50.5 ± 8.6 pg/mL.

298 Although the values widely ranged, it was within the range of concentration in previous reports of OA
299 knees. Meanwhile, the mean IL-6 concentration was 20.7 ± 3.8 pg/mL at the time of plate removal, which
300 was similar or possibly higher than normal concentration. These observations suggest that joint
301 inflammation can be reduced after AKO, but there may be some residual inflammation.

302 Synovial angiogenesis has been suggested as a characteristic finding of OA synovitis.[50,51] In the present
303 study, synovitis was reduced after AKO, in association with reduced synovial angiogenesis in the
304 subintimal layer, as assessed by the number of VEGF-positive luminal structures. Although the
305 mechanisms underlying the reduction in angiogenesis are currently unknown, it appears to be a secondary
306 consequence of reduced inflammation. Meanwhile, the ratio of IL-1β- and IL-6-positive cells in the entire
307 synovium did not significantly change before and after AKO. However, the ratios of IL-1β- and IL-6-
308 positive cells were significantly reduced postoperatively when the intimal layer was assessed separately.
309 As multilayering of the intima is the most typical finding in OA synovium,[4,11] changes in the intima may
310 sensitively reflect the joint condition and synovitis. Since inflammatory cytokines such as IL-1β and tumor
311 necrosis factor-α induce the expression of collagenase and aggrecanase in cartilage,[52,53] thereby the
312 reduced inflammatory cytokine expression in the synovial intima may lead to reduction in cartilage
313 catabolic responses. Further study is required to elucidate the interaction between cartilage and synovium.

314 Studies have shown that the polarity of synovial macrophages is closely associated with the development

315 of OA. Zhang et al.[10] found that in a mouse model of OA, macrophages in the synovium and joint space
316 aggregated, increasing M1 synovial macrophages and promoting OA progression. It has also been shown
317 that M1 macrophages increase and M2 macrophages decrease in the synovium during OA development.[9]
318 In the present study, synovitis score was improved in association with decreased macrophage infiltration,
319 and M1/M2 polarity shifted from M1 dominant state toward M2 after surgery, suggesting that macrophage
320 status played a role in the improved synovitis. Recently, it has been reported that a distinct subset of the
321 macrophage population residing in the synovium plays different roles in joint homeostasis and
322 inflammation. Membrane-forming macrophages selectively express CX_3CR1 and form a dense barrier
323 between the synovium and joint cavity, whereas CX_3CR1-negative macrophages proliferate in association
324 with disruption of the barrier in responded to inflammation.[54] In the present study, a disorganized
325 arrangement of CX_3CR1-positve synovial lining cells was observed preoperatively, whereas CX_3CR1-
326 positve synovial cells were well aligned in the superficial layer of the synovium in the samples obtained
327 at the time of second-look arthroscopy. Therefore, these observations suggested that not only the
328 macrophage phenotype but also the macrophage-forming microstructure of the synovium were improved
329 to a more physiological state in association with reduced inflammation.
330
331 **Limitations**
332 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.

341 **Table 1.** Patient demographics and baseline characteristics[a]

Characteristics		
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	12
	Mosaic plasty	1
	Meniscectomy	4
	Meniscal repair	7
Period from AKO to plate removal (months)		16.8 ± 1.5 (range, 11–38)

342 [a]OWHTO, opening wedge high tibial osteotomy; DTO, distal tuberosity osteotomy; DLO, double level

343 osteotomy; AKO, around-knee osteotomy.

344

Table 2. Radiographic changes and patient-reported outcomes before and after surgery[a]

		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]WBL, weight-bearing line; KSS, the Knee Society score; KOOS, the knee injury and osteoarthritis outcome score; ADL, activity of daily living; QOL, quality of life; IKDC, the International Knee Documentation Committee.

[b]Statistical significance: $P < .05$.

Table 3. Changes in SF and synovium before and after surgery[a]

		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]SF, synovial fluid; pts, points; IL, interleukin; HPF, high-power field; Preop, preoperative; Postop, postoperative.

[b]Data are expressed as mean ± SEM

[c]Statistical significance: $P < .05$.

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

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