Under-correction was associated with residual proinflammatory Cytokine gene expressions after high tibial osteotomy

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Disclosures: Kohei Nishitani (8-Editorial Review Board of JOR), Shigeo Yoshida (N), Shinichi Kuriyama (5- Olympus Terumo Biomaterials), Shinichiro Nakamura (N), Yu Kobori (N), Koichi Murata (N), Hiroyuki Yoshitomi (N), Takayuki Fujii (N), Shuichi Matsuda (N) (Information for disclosures can be taken from the online abstract system after entering ALL authors.)

INTRODUCTION:
Medial open wedge high tibial osteotomy (MOWHTO), which changes lower limb alignment from varus to valgus, is widely performed for medial knee osteoarthritis (OA). Although biomechanical findings have shown that MOWHTO decreases medial compartment loading, whether joint inflammation in the biological microenvironment are altered by optimizing knee alignment has not fully elucidated yet. Previously, the reduction of gene expressions of pro-inflammatory cytokines and chemokines, and the polarity change of synovial macrophages from M1 to M2 in synovial tissues (STs) after MOWHTO were reported [1]. However, the relationship between alignment correction and synovial gene expressions is still unclear. Thus, the purpose of this study was to evaluate the relationship between pre- and postoperative knee alignment and synovial gene expression, and to find the cutoff point of postoperative knee alignment where gene expressions of synovial inflammation improved.

METHODS:
Patients: This investigation was approved by the institutional review board, and all patients provided written informed consent to participate. This study included consecutive patients with medial compartment OA of the knee who underwent MOWHTO between June 2018 and May 2020. All patients were followed-up postoperatively until the time of plate removal approximately one year to assess of clinical and radiographic outcomes.
Clinical and radiological evaluation: Clinical evaluation was carried out using knee injury and osteoarthritis outcome scores (KOOS) preoperatively and postoperatively (at the time of plate removal). For radiographic assessment, the weight bearing line ratio (WBLR) (<50% indicates varus alignment) and the hip-knee-ankle angle (HKAA) (positive value indicates varus alignment) were measured with anteroposterior whole-leg standing radiography. Knee OA was graded using the Kellgren-Lawrence (KL) classification.
Surgical procedure and sample collection: An initial arthroscopic examination was routinely performed prior to MOWHTO. Synovial tissues (STs) were harvested from the suprapatellar pouch during arthroscopic examination. MOWHTO was performed with target WBLR of 60-62.5%[2]. Then the osteotomy was fixed with TomoFixTM Medial High Tibial Plate (DePuy Synthes). Plate removal was performed about one year after OWHTO when bone healing was confirmed, and STs were collected from suprapatellar pouch during arthroscopic examination prior to plate removal.
Gene expression analysis in synovial tissue: Total RNA was extracted and purified from fresh STs samples followed by reverse transcription. Then, SYBR Green real-time PCR (TOYOBO) was performed using ABI Step One Plus RT-PCR System (Thermo Fisher Scientific) in triplicate for each sample to determine relative gene expression using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping control using the $2^{-\Delta\Delta Ct}$ method. The gene expression was determined in all STs for 10 genes previously reported to be associated with the pathogenesis of OA[1].
Statistical analysis: Between group differences were evaluated using the Mann-Whitney U test for continuous variables and the Fisher's exact test for categorical variables. Corelations between lower limb alignments and synovial gene expressions were determined using Spearman rank correlations. A receiver operating characteristic curve (ROC) analysis was used to determine the cut-off values of WBLR for improvement of gene expression from preoperative to postoperative. $P < 0.05$ was considered as significant. A priori sample size calculation was performed using G*Power 3.1.9.6 (Heinrich-Heine-Universität Düsseldorf), with power = 0.80, alpha error = 0.05, and correlation coefficient = 0.45 (95% confidence interval: 0–0.75) required n = 33.

RESULTS SECTION:
A total of 40 patients were enrolled, and four patients without plate removal were excluded. The mean age of analyzed 36 patients was 61.4 ± 7.1 years, and sex was female dominant (male 16, female 20). In radiographic evaluation, preoperative KL grades were 1 in 3, 2 in 10, 3 in 17, and 4 in 6 knees. Mean preoperative HKAA was 8.3 ± 4.2° varus, which was corrected to 0.6 ± 2.6° valgus, postoperatively. Pre- and postoperative WBLR was 14.6 ± 16.2% and 51.4 ± 9.4 %, respectively. Plates were removed 13.9 ± 3.7 months after OWHTO. Postoperative HKAA was correlated with *IL1B* ($\rho = 0.47$, $p = 0.004$) and *IL6* ($\rho = 0.42$, $p = 0.01$), and postoperative WBLR also correlated with *IL1B* ($\rho = -0.43$, $p = 0.008$) and *IL6* ($\rho = -0.41$, $p = 0.01$), indicating knees with postoperative under-correction associated with high expression of proinflammatory cytokines. In ROC analysis, ROC curves for postoperative WBLR to postoperative improvement of gene expression of *IL1B* and *IL6* were drawn (Fig 1) with AUCs of 0.62 ($p = 0.27$) and 0.74 ($p = 0.03$), respectively. The cutoff value to predict improvement of *IL6* was postoperative WBLR = 52%. There were significant differences in postoperative proinflammatory cytokine gene expressions between the patients with WBLR <52% and >52% (Fig 2). Patients with WBLR <52% showed inferior postoperative every KOOS scale to those with WBLR >52%. When pre-operative WBLR >0% (n = 30), postoperative WBLR was <52% in 11 cases and >52% in 19 cases. However, when preoperative WBLR <0% (n =6), no patients achieved postoperative WBLR >52% ($p = 0.006$) (Fig. 3).

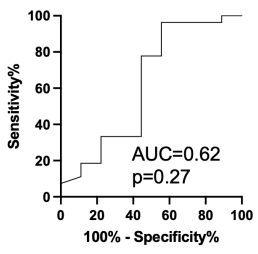
DISCUSSION:
From this study, relationship between knee alignment and gene expressions were observed after MOWHTO, and patients with under-correction tended to have insufficient improvement of proinflammatory cytokine expressions. The postoperative alignment to predict improvement of *IL6* gene expression was WBLR = 52%, which was considered as the minimum postoperative alignment for biological improvement. When patients were divided into two group by this value, patients with WBLR <52% showed residual higher proinflammatory cytokine expressions and inferior clinical scores. To achieve this alignment, preoperative WBLR should be more than 0%.

SIGNIFICANCE/CLINICAL RELEVANCE:
This study recommended postoperative WBLR > 52% after MOWHTO for the biological improvement in proinflammatory gene expressions in STs.

REFERENCES:
1. Yoshida S, Nishitani K, et al. Arthritis Rheumatol. 2023 Jun;75(6):950-960. 2. Kuriyama S, Nishitani k, et al. J Orthop Res. 2019 Apr;37(4):898-907.

IMAGES AND TABLES:

Fig. 1 ROC for *IL1B* improvement



ROC for *IL6* improvement

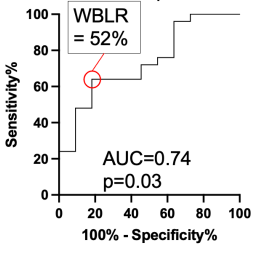


Fig. 2 *IL1B* *IL-6*

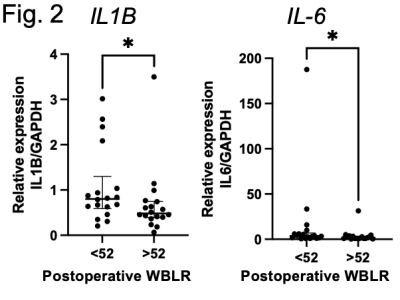


Fig. 3