

Inflammatory and Extracellular Matrix Signatures Associated with Pre-Operative Knee Stiffness and Post-Operative Arthrofibrosis Following Total Knee Arthroplasty in Osteoarthritis Patients

Anne Oerlemans^{1,2}, Izzat Zulkiflee¹, Kieran Joyce³, Catriona Flaherty³, Gerard Sheridan^{3**}, Isma Liza Mohd Isa^{1,2,4**}

¹CÚRAM Research Ireland Centre for Medical Devices, University of Galway, Ireland, ²Pharmacology and Therapeutics, School of Medicine, University of Galway, Ireland, ³Department of Orthopaedics, University of Galway, Ireland, ⁴Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia

* ismaliza.mohd@universityofgalway.ie; ** gerard.g.sheridan@universityofgalway.ie

Disclosures: None

INTRODUCTION: Arthrofibrosis (AF) is a debilitating disorder occurring in up to 10% of patients following total knee arthroplasty (TKA), resulting in pain and restricted range of motion (ROM). The pathogenesis involves chronic inflammation and excessive extracellular matrix (ECM) deposition; however, early molecular predictors remain poorly defined. This study aimed to identify inflammatory and ECM gene expression signatures associated with pre-operative knee stiffness and post-operative arthrofibrosis in osteoarthritis (OA) patients undergoing TKA.

METHODS: Synovial tissue was collected from twelve osteoarthritis patients (n = 9 with pre-operative stiffness; n = 3 without) undergoing total knee arthroplasty (TKA) at Merlin Park and University Hospital Galway. The cohort included both males and females (67% male). All participants provided written informed consent. RNA was extracted and analysed by quantitative RT-PCR (qRT-PCR) for inflammatory (IL-6, IL-17A, TNF- α , CCL2) and extracellular matrix (ECM) markers (ACTA2, TGF- β 1, CTGF, PDGF-B, COL1A2). Clinical data (Oxford Knee Score [OKS], Range of Motion [ROM], EQ-5D) were correlated with molecular results using Spearman correlation and Mann-Whitney U tests.

RESULTS: Twelve osteoarthritis patients were included (n = 9 with pre-operative stiffness; n = 3 without). Patients with stiffness were generally older (median 72 years) and predominantly male (67%). Pre-operative stiffness was associated with significantly increased IL-6 expression (2.3-fold; p = 0.03), while IL-17A (1.8-fold), ACTA2 (1.7-fold), and TGF- β 1 (1.5-fold) showed non-significant upward trends with large effect sizes (r = 0.6–0.7) (Figure 1). Correlation analysis revealed positive relationships between inflammatory and ECM markers across both stiffness and non-stiffness groups (r = 0.19, p = 0.63) (Figure 2). ACTA2 and TGF- β 1 expression showed moderate negative correlations with range of motion (r = -0.75) and Oxford Knee Score (r = -0.68). Global expression patterns of fibrotic ECM markers (ACTA2, TGF- β 1) were more pronounced than inflammatory markers (IL-17A, TNF- α), indicating fibrotic dominance in arthrofibrotic tissue. Similar expression and correlation trends were observed in patients who developed post-operative arthrofibrosis.

DISCUSSION: The observed upregulation of IL-6 and IL-17A suggests that inflammatory signalling precedes fibrotic activation in the synovium, potentially driving increased ACTA2 and TGF- β 1 expression in patients predisposed to arthrofibrosis. The small sample size limits statistical power, but the consistent expression patterns support biological relevance. These findings provide mechanistic insight into how inflammation contributes to fibrotic tissue remodelling and loss of joint mobility in post-TKA patients.

SIGNIFICANCE: Understanding the link between inflammatory and fibrotic gene activity may help identify patients at risk of developing arthrofibrosis following TKA. The identification of IL-6, IL-17A, ACTA2, and TGF- β 1 as potential biomarkers could inform early diagnostic screening and guide the design of targeted anti-fibrotic therapies.

REFERENCES: Chen et al. (2021). *EbioMedicine*, 70: 103486. Usher et al. (2019). *Bone Research*, 7: 9.

ACKNOWLEDGEMENTS: This study received ethical approval from the Clinical Research Ethics Committee of Galway University Hospitals (C.A. 3246). It was funded by the Health Research Board, Ireland, under the Emerging Investigator Award for Health (EIA-2022-010).

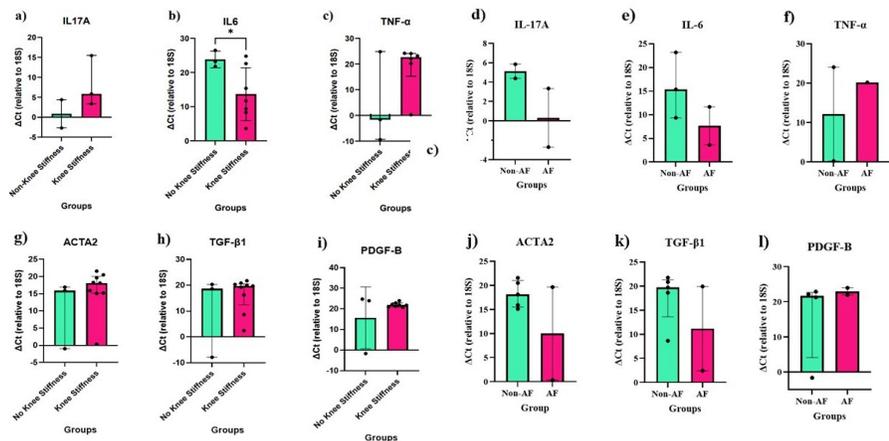


Figure 1. Differential expression of inflammatory and fibrotic markers in pre-operative stiffness and arthrofibrosis. (a–c) Inflammatory markers (IL-17A, IL-6, TNF- α) and (g–i) fibrotic markers (ACTA2, TGF- β 1, PDGF-B) in patients with and without pre-operative stiffness. (d–f, j–l) Corresponding expression patterns in post-operative arthrofibrosis (AF) vs non-AF synovium. Bars show mean \pm SD of Δ Ct (relative to 18S). Lower Ct scores indicate higher gene expression. *p < 0.05 for IL-6 between stiffness groups (unpaired t-test).

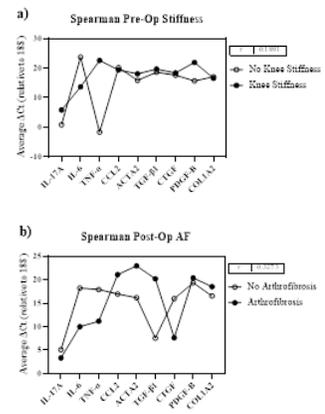


Figure 2. Correlations between gene expression and clinical outcomes. (a) Spearman correlations between Δ Ct expression and pre-operative stiffness. (b) Correlations between Δ Ct expression and post-operative arthrofibrosis. Positive r-values indicate increased expression with reduced ROM and OKS.