Senescence Biomarkers in the Blood of Anterior Cruciate Ligament (ACL) Reconstruction Patients

Noah Lawson[1], Brian Humphrey[1], Ethan Starr[1], Michael J Aderman, MS[2,3], Steven Malvasi, MS[2], Jessica Curtin[1], Daniel Gordon, MS[1], Jesse Trump, MS[1], Kenneth L. Cameron, PhD[2,3], Kevin J. O'Donovan, PhD[1].

1. Department of Chemical and Biological Science and Engineering, United States Military Academy, West Point, NY.
2. John A. Feagin Sports Medicine Fellowship, Keller Army Hospital, United States Military Academy, West Point, NY.
3. Uniformed Services University of Health Sciences Department of Physical Medicine & Rehabilitation, Bethesda, MD.

Introduction

- Post-traumatic osteoarthritis (PTOA) develops following traumatic joint injuries and is a major cause of disability in both civilian and military populations.
- Anterior cruciate (ACL) injuries are among the strongest risk factors for PTOA and occur at significantly higher rates in military personnel.
- Cellular senescence contributes to chronic inflammation through the senescence associated secretory phenotype (SASP), which may accelerate cartilage degeneration following injury.
- Previous studies have primarily evaluated senescence biomarkers in synovial fluid and cartilage, while systemic blood-based biomarkers remain poorly characterized
- Peripheral blood mononuclear cells (PBMCs) may provide a minimally invasive method to evaluate systemic senescence signaling after ACL injury.

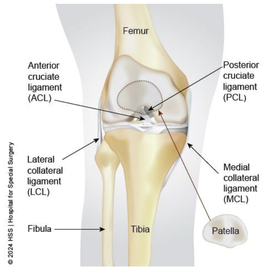

Figure 1. Anterior view of the human knee joint illustrating the major stabilizing ligaments, including the ACL, PCL, MCL, and LCL), along with associated osseous structures (femur, tibia, fibula, and patella).

Methods

Study Design
- Prospective cohort study of ACL reconstruction patients and matched healthy controls.
- Participants recruited from the United States Military Academy (USMA) and USMAPS.
- Samples collected at:
  - Acute injury (≤15 days post-injury)
  - Two years following ACL reconstruction

Population
- 10 male participants:
  - 5 ACL-injured subjects
  - 5 matched controls
- Controls matched by sex, age, height, and weight

Biomarker Analysis
- PBMCs isolated from whole blood samples.
- RNA extracted and reverse transcribed to cDNA.
- RT-qPCR performed using TaqMan assays.
- Sixteen genes analyzed in three categories:
  - Inflammatory markers
  - Cell cycle/senescence markers
  - Extracellular matrix remodeling markers
- Relative expression calculated using the Pfaffl method and normalized to RNA45S.
- Cohen's D effect size calculations were used to quantify the difference between case and control groups as well as case versus case across time points

Results

Gene Name	Function
IL6	proinflammatory cytokine, correlates with osteoarthritis severity
TNFA	inflammatory cytokine made by macrophages/monocytes during acute inflammation
IL1B	pyrogenic cytokine
IFNG	cytokine involved in innate/adaptive immunity, primary activator of macrophages.
CD14	immune system protein, detects bacteria
CDKN2A	tumor suppressor, cellular senescence marker
CDKN1A	cyclin kinase inhibitor, cellular senescence marker
TP53	tumor suppressor, cell cycle arrest
GLB1	indicator of senescent phenotype
COL5A1	Type V collagen, contribute to bone and interstitial matrix
ENG	TGF beta co-receptor, involved in chondrogenesis
TIMP1	Metalloproteinase (MMP9) inhibitor, degradation of extracellular matrix
PRG4	Chondrocyte proteoglycan
TIMP2	Metalloproteinase (MMP2) inhibitor, degradation of extracellular matrix
CHAD	Chondroadherin, cartilage matrix protein
MMP9	Matrix metalloproteinase, collagenase, wound repair

Table 1: Selected SASP and collagen-related target genes and their biological function test

Cohen's d effect Size

•Several biomarkers demonstrated substantial differential expression between ACL reconstruction cases and controls, as well as significant longitudinal changes between the acute post-injury period (Time A) and two-year follow-up (Time E).
•**MMP9** exhibited the largest and most consistent effect sizes across comparisons, showing strong separation between cases and controls at both time points and significant longitudinal changes, indicating persistent alterations in extracellular matrix remodeling following ACL injury.
•**IL1B**, **CD14**, and **COL5A1** demonstrated relatively modest case-control differences but large longitudinal effect sizes, suggesting these markers are more reflective of temporal changes in the post-injury biological environment than acute injury status alone.
•**CDKN2A (p16)** and **ENG** showed strong effects at Time A, indicating activation of senescence-related and angiogenic pathways during the early post-injury period. ENG effects diminished at later time points, whereas CDKN2A remained altered longitudinally.
•**CHAD** and **PRG4** displayed larger effect sizes at Time E than at Time A, suggesting involvement in long-term cartilage remodeling and joint homeostasis rather than acute injury responses.
•**IL6** demonstrated moderate-to-large effect sizes across both acute and chronic comparisons, indicating sustained inflammatory signaling throughout recovery.
•In contrast, **TP53**, **IFNG**, and **TNFA** maintained relatively small effect sizes across comparisons, indicating stable expression and supporting their potential utility as reference or control biomarkers within this cohort.
•Collectively, these findings identify distinct biomarker patterns associated with acute injury response, longitudinal recovery, and long-term joint remodeling following ACL reconstruction.

Cohen's d Effect Size

	IL6	TNFA	IL1B	CD14	IFNG	CDKN1A	CDKN2A	TP53	GLB1	COL5A1	ENG	TIMP1	PRG4	TIMP2	CHAD2	MMP9
ALL (case/control)	-0.472	-0.096	0.0613	0.0435	0.0351	-0.12972	-0.52622	-0.355	-0.037	-0.0399	-0.321	-0.069	-0.552	-0.084	-0.679	1.154
Case (A)/Case (E)	-0.634	-0.76	-1.916	-1.384	-0.297	0.30301	-0.96839	-0.213	-0.722	-1.4298	0.1388	-0.807	0.1229	-0.81	-0.2135	-1.735
Case/control A	-0.522	-0.111	0.1981	0.036	0.1481	0.1493	-1.12586	-0.177	0.2282	-0.235	-1.136	0.0027	0.0244	-0.259	0.0528	0.8579
Case/control E	-0.533	-0.12	0.0726	0.0757	-0.007	-0.59418	-0.20932	-0.486	-0.217	0.03631	-0.216	-0.119	-0.996	-0.017	-1.3346	2.0825
Control(A)/Control(E)	0.007	0.0061	0.0188	0.049	0.0042	0.00525	0.00309	0.0054	0.014	0.00941	-0.003	0.0153	0.0095	0.009	0.0198	0.0089

Figure 2. Heatmap displaying Cohen's *d* effect sizes for biomarker expression across cross-sectional and longitudinal comparisons between ACL reconstruction patients and healthy controls. Comparisons include overall case-control differences, acute post-injury (Time A) and two-year follow-up (Time E) case-control analyses, and temporal changes within case and control cohorts.

Acute Injury Response
•Four genes demonstrated significant upregulation at the time of injury:
- **IL6**
- **CDKN1A**
- **CDKN2A**
- **COL5A1**

•Findings indicate activation of inflammatory, senescence, and tissue repair pathways immediately following ACL injury.

Two-Year Follow-Up
- **IL6** remained elevated two years after reconstruction, suggesting persistent inflammatory signaling.
- **PRG4** expression was significantly increased at two years post-reconstruction.
- **MMP9** expression decreased by more than two-fold at the two-year time point.

Overall Findings
- Senescence-associated biomarkers were detectable in PBMCs using qPCR.
- Distinct gene expression signatures persisted long after surgical reconstruction.
- Results support a systemic biological response to ACL injury that may contribute to PTOA development.

	Fold Change at Time of Injury				Fold Change at 2 year mark			
	Control*	Injured	SEM	Gene	Control*	Injured	SEM	Gene
Inflammation	1	**4.11**	1.87	IL6	1	**2.01**	0.71	IL6
	1	1	0.5	TNFA	1	1.22	0.39	TNFA
	1	0.99	0.5	IL1B	1	0.88	0.19	IL1B
	1	1.5	0.75	CD14	1	1.05	0.25	CD14
	1	0.98	0.49	IFNG	1	1.85	1.18	IFNG
Cell cycle/senescence	1	**3.94**	1.97	CDKN2A	1	1.91	0.66	CDKN2A
	1	**3.12**	1.56	CDKN1A	1	1.93	0.76	CDKN1A
	1	1.1	0.55	TP53	1	1.61	0.37	TP53
	1	0.92	0.46	GLB1	1	1.3	0.29	GLB1
Extracellular matrix	1	**3.7**	1.85	COL5A1	1	1.76	0.68	COL5A1
	1	1.81	0.91	ENG	1	1.75	0.47	ENG
	1	1.22	0.61	TIMP1	1	1.18	0.19	TIMP1
	1	1.07	0.53	PRG4	1	**3.47**	0.82	PRG4
	1	1.06	0.53	TIMP2	1	1.09	0.21	TIMP2
	1	0.9	0.45	CHAD	1	1.93	1.49	CHAD
	1	0.83	0.41	MMP9	1	**0.44**	0.11	MMP9

* Control values normalized to one. See Methods.

Table 2: Summary Data for qPCR Analysis using fold change at time of injury and 2 years post reconstruction. All control values normalized to a value of 1.

Conclusions

- ACL Injury is associated with measurable changes in senescence associated gene expression within peripheral blood mononuclear cells
- Elevated expression of IL6, CDKN1A, and CDKN2A suggests activation of inflammatory and cellular senescence pathways following injury
- Persistent biomarker alterations at two years post reconstruction indicate that biological changes continue long after clinical recovery and unrestricted return to duty
- PBMC-based biomarker analysis represents a minimally invasive approach for monitoring PTOA risk.
- Future studies with larger cohorts and longer follow-up are needed to validate these biomarkers and assess their predictive value for PTOA development

References

- **Ok Hee Jeon , Chaekyu Kim, Remi-Martin Laberge, Marco Demaria et al**. *Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment.* Nat Med, 2017 Jun;23(6):775-781. https://doi.org/10.1038/nm.4324.
- **Dilley JE, Bello MA, Roman N, McKinley T, Sankar U.** *Post-traumatic osteoarthritis: A review of pathogenic mechanisms and novel targets for mitigation.* **Bone Reports.** 2023;18:101658. https://doi.org/10.1016/j.bonr.2023.101658
- **Cameron KL, Driban JB, Svoboda SJ.** *Osteoarthritis and the Tactical Athlete: A Systematic Review.* **Journal of Athletic Training.** 2016;51(11):952–961. https://doi.org/10.4085/1062-6050-51.5.03
- **Coryell PR, Diekman BO, Loeser RF.** *Mechanisms and therapeutic implications of cellular senescence in osteoarthritis.* **Nature Reviews Rheumatology.** 2021;17(1):47–57. https://doi.org/10.1038/s41584-020-00533-7
- **Waltz RA, Whitney KE, Duke VR, et al.** *A Systemic and Local Comparison of Senescence in an Acute Anterior Cruciate Ligament Injury—A Pilot Case Series.* **Life (Basel).** 2023;13(7):1567. https://doi.org/10.3390/life13071567
- **Svoboda SJ, Harvey TM, Owens BD, et al.** *Changes in serum biomarkers of cartilage turnover after anterior cruciate ligament injury.* **American Journal of Sports Medicine.** 2013;41(9):2108–2116.

Acknowledgments

The authors would like to thank Dr. Loverde and the CBSE laboratory staff for their support, as well as Keller Army Community Hospital for its collaboration. We also gratefully acknowledge the contributions of R. Dolph, J. Stearns, J. Ives, K. Rook, and M. Yang to this project.