Plasma Inflammation Characterization of Hemarthrosis After Traumatic Knee Joint Injury in Military Personnel

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Introduction

- Cases of osteoarthritis (OA) are a common cause of disability among medically separated military service members and rates of OA in the military have been observed at higher rates than the general population.
- Emerging evidence has revealed an association between intra-articular soft tissue injuries and cases of OA resulting in total joint replacements.
- These acute, traumatic soft-tissue injuries typically result in significant hemarthrosis containing inflammatory biochemicals and cells associated with OA.
- To attain a better understanding of the OA development process, the purpose of this study was to determine the expression profile of inflammatory cytokines in the patient plasma near the time of injury.

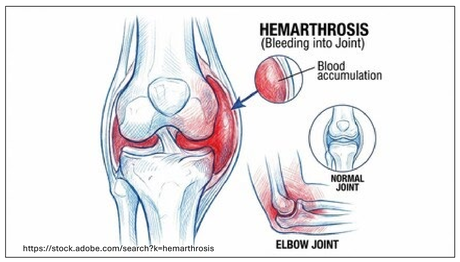

Figure 1. Knee hemarthrosis is the accumulation of blood within the knee joint, usually caused by trauma, ligament injury (e.g.,ACL tear), fracture, or a bleeding disorder. It typically presents with rapid swelling, pain, stiffness, and reduced range of motion as blood fills and distends the joint space.

Methods

- A prospective case-series study design was conducted among Cadets enrolled at a US Service Academy.
- Potential subjects with a knee joint injury were referred to and screened by a military orthopaedic surgeon to determine eligibility for the study.
- Subjects underwent informed consent and provided demographic and injury history information. In addition to an aspiration of the knee during initial evaluation, blood plasma samples were collected within 96 hours of injury.
- Plasma samples were collected, stored, and batch-screened by ELISA to probe for the presence of a panel of pro- and anti-inflammatory cytokines and select other blood-based biomarkers.

Results

Function	Biomarker	Role in Knee Hemarthrosis
Pro-inflammatory biomarkers	IL-1α	Early pro-inflammatory cytokine released from damaged joints; promotes synovitis and cartilage-degrading enzymes.
	IL-1β	Key driver of blood-induced joint damage; stimulates cartilage degradation and MMP production.
	IL-6	Marker of acute joint inflammation and synovial activation after bleeding.
	TNF-α	Amplifies synovitis, cartilage breakdown, and osteoclast activation.
	IFN-γ	Reflects activation of T cells and macrophages; contributes to chronic inflammation.
Immune-cell recruitment/chemotaxis	IL-8	Neutrophil chemoattractant that recruits inflammatory cells into the joint.
	MCP-1	Recruits monocytes/macrophages to the joint, indicating persistent inflammation.
	MIP-1	Attracts leukocytes and macrophages to the synovium, promoting inflammation.
Cartilage degradation	IL-1α	Early pro-inflammatory cytokine released from damaged joints; promotes synovitis and cartilage-degrading enzymes.
	IL-1β	Key driver of blood-induced joint damage; stimulates cartilage degradation and MMP production.
	TNF-α	Amplifies synovitis, cartilage breakdown, and osteoclast activation.
	MMP-3	Marker of cartilage and extracellular matrix degradation.
Repair and remodeling	TGF-β1	Regulates tissue repair and fibrosis; associated with synovial hypertrophy and remodeling.
	Osteopontin	Involved in inflammation, tissue remodeling, and bone turnover.
	Osteocalcin	Marker of bone formation and subchondral bone remodeling.
Anti-inflammatory /protective	IL-1Ra	Natural inhibitor of IL-1 signaling; protective against inflammation and cartilage damage.
	IL-10	Anti-inflammatory cytokine that suppresses pro-inflammatory mediator production.
	α2-Macroglobulin	Protease inhibitor that neutralizes MMPs and limits cartilage damage.
Angiogenesis and recurrent bleeding	VEGF-A	Promotes angiogenesis and formation of fragile vessels that may contribute to recurrent bleeding.

Table 1. Functional classification and biological roles of biomarkers associated with knee hemarthrosis. Biomarkers are grouped according to patho-physiology of blood-induced joint injury.

Biomarker	Median (range)	Unit	Observation
IL-1α	4.36 (1.72–29.11)	pg/mL	Patient 15 highest
IL-1β	4.92 (0.54–15.37)	pg/mL	Patient 7 highest
IL-6	4.92 (0.54–15.37)	pg/mL	Patient 7 highest
TNF-α	34.02 (18.89–63.88)	pg/mL	Detected in all; patient 6 highest
IFN-γ	11.24 (2.14–68.97)	pg/mL	Patient 9 markedly elevated
IL-8	0 (0–0)	pg/mL	Undetected
MCP-1	25.55 (13.74–95.16)	pg/mL	Patient 4 highest
MIP-1	31.13 (4.72–71.89)	pg/mL	Broad interpatient variability
IL-1α	4.36 (1.72–29.11)	pg/mL	Patient 15 highest
IL-1β	4.92 (0.54–15.37)	pg/mL	Patient 7 highest
TNF-α	34.02 (18.89–63.88)	pg/mL	Detected in all; patient 6 highest
MMP-3	1.33 (0.46–2.25)	ng/mL	Relatively narrow distribution
TGF-β1	373.40 (48.56–1514.79)	pg/mL	Patients 6 and 14 highest
Osteopontin	24.95 (18.72–32.77)	ng/mL	Relatively stable
Osteocalcin	8.08 (2.25–19.33)	ng/mL	Broad range without one extreme value
IL-1Ra	92.89 (0–539.17)	pg/mL	Zero in 5/15; patient 6 highest
IL-10	0 (0–86.06)	pg/mL	Zero in 9/15; patient 4 highest
α2-Macroglobulin	1097.30 (802.68–1319.40)	ng/mL	Relatively stable
VEGF-A	0 (0–112.96)	pg/mL	Zero in 10/15; patient 6 highest

Table 2. Summary of biomarker concentrations measured in plasma samples from 15 patients, grouped according to their principal biological functions. Values are presented as median concentrations with ranges. Observations highlight notable findings. Concentrations are reported in pg/mL or ng/mL as indicated.

Pro-Inflammatory Burden

- We ranked the 15 patients from highest to lowest relative pro-inflammatory biomarker burden within this cohort using only pro-inflammatory markers IL-1α, IL-1β, IL-6, TNF-α, IFN-γ, MCP-1, and MIP-1.
- For each included marker, patients were assigned an average percentile rank. The seven percentile ranks were averaged and multiplied by 100. Highest quartile, middle 50%, and lowest quartile are cohort-relative categories.
- IL-8 was excluded and anti-inflammatory markers were not factored in for this analysis.

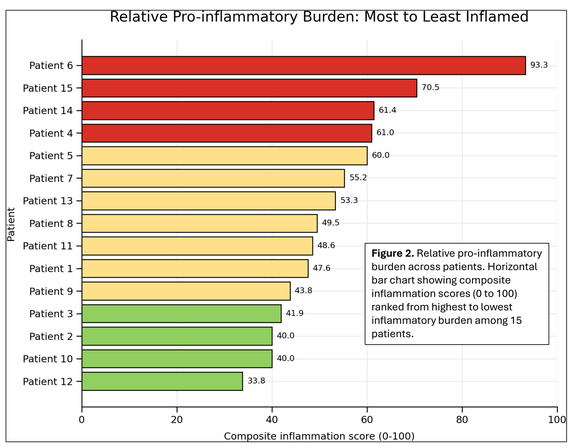

Figure 2. Relative pro-inflammatory burden across patients. Horizontal bar chart showing composite inflammation scores (0 to 100) ranked from highest to lowest inflammatory burden among 15 patients.

Conclusions

- **Pro-inflammatory activity is broadly present**
  - IL-1α, IL-1β, IL-6, TNF-α, IFN-γ, MCP-1, and MIP-1 were measurable in every patient. TNF-α was comparatively consistent, with a median of 34.02 pg/mL, while IL-1α, IFN-γ, and MCP-1 showed pronounced patient-specific peaks.
- **Anti-inflammatory compensation is inconsistent**
  - IL-1RA was measurable in 10 of 15 patients.
  - IL-10 was measurable in 6 of 15 patients.
  - TGF-β1 was measurable in all patients but varied approximately 31-fold, from 48.56 to 1514.79 pg/mL.
  - The results suggest that inflammatory activity is not accompanied by a uniform counter-regulatory response.
- The elevation of IL-10, IL-1RA, and TGF-β1 suggests some patients may be exhibiting inflammation resolution and joint homeostasis, while others may remain in a more persistent inflammatory state.
- The dataset is consistent with a heterogeneous inflammatory response following hemarthrosis, accompanied by variable counter-regulatory, angiogenic, matrix-remodeling, and bone-turnover signals.
- VEGF-A was zero in 10 of 15 patients. Patient 6 showed the highest detectable VEGF-A and the broadest multi-marker response.

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Acknowledgments

The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the Uniformed Services University or the Department of War.
Conflict of Interest Statement: The authors have no financial interests or relationships to disclose.
MIRROR Acknowledgment Statement: This work was funded in part by the Musculoskeletal Injury Rehabilitation Research for Operational Readiness (MIRROR), Department of Physical Medicine & Rehabilitation, Uniformed Services University, Bethesda, MD, (HU00011920011).