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Immunological Profile and Local Inflammatory Mechanisms in Posttraumatic Hemarthrosis Following Intra-Articular Fractures of the Lower Limbs

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ABSTRACT

Background: *Intra-articular fractures of the lower extremities are frequently complicated by posttraumatic hemarthrosis (HA), which contributes to the development of persistent inflammation, secondary synovitis, and joint dysfunction. Recent evidence highlights the role of immune mechanisms in the pathogenesis of joint injury, yet the dynamics and predictive value of immunological parameters in HA remain insufficiently characterized.*

Materials and Methods: *This prospective clinical-laboratory study included 150 individuals: 60 patients with HA due to intra-articular fractures (main group), 60 with isolated HA without fracture (comparative group), and 30 healthy controls. Systemic and synovial levels of lymphocyte subpopulations, immunoglobulins, circulating immune complexes (CIC), cytokines (IL-6, IL-1β, TNF-α, IL-10), matrix metalloproteinase-9 (MMP-9), and lysozyme were assessed using flow cytometry, ELISA, nephelometry, and functional assays.*

Results: *Patients with fracture-associated HA demonstrated significantly greater immune dysregulation compared to other groups, including reduced $CD4^+$ and $CD8^+$ T cells, increased NK cells and CIC, and elevated IL-6, IL-1β, and MMP-9 levels both systemically and in synovial fluid. Synovial cytokine levels strongly correlated with pain severity, joint effusion volume, and need for repeated aspirations.*

Conclusion: *HA in the setting of intra-articular fractures is characterized by a distinct local and systemic inflammatory immune profile. The findings support the use of immune markers—particularly IL-6, $CD4^+$, CIC, and MMP-9—as indicators of inflammatory burden and potential predictors of adverse outcomes.*

Keywords: *Hemarthrosis, intra-articular fracture, cytokines, T cells, synovial fluid, MMP-9, immune response, inflammation.*

INTRODUCTION

Intra-articular fractures of the lower limbs are among the most challenging forms of skeletal trauma due to their frequent complication with hemarthrosis (HA), which contributes to prolonged inflammation, synovial fibrosis, and irreversible joint dysfunction [1,2]. The high incidence of HA following intra-articular fractures—reported in up to 60–70% of such injuries—has prompted renewed interest in its underlying biological mechanisms [3].

Hemarthrosis triggers a cascade of immunoinflammatory events within the joint. Blood components entering the synovial cavity activate innate immunity via damage-associated molecular patterns (DAMPs), initiating neutrophil infiltration, macrophage polarization, and release of matrix metalloproteinases (MMPs), particularly MMP-9 [4,5]. Simultaneously, adaptive immunity is recruited: $CD4^+$ T cells, $CD8^+$ cytotoxic lymphocytes, B cells, and NK cells contribute to the immune response, while dysregulated cytokine profiles—particularly elevated IL-6, IL-1β, and TNF-α—amplify the inflammatory process [6,7].

While such immune responses are well-characterized in chronic joint diseases, including rheumatoid arthritis and osteoarthritis [8], their role in acute posttraumatic settings remains less explored. Moreover, there is limited understanding of the immunological distinction between fracture-associated and non-fracture hemarthrosis.

Several studies have emphasized the prognostic importance of immune markers such as IL-6, $CD4^+$ lymphocytes, CIC, and MMP-9 in joint inflammation and remodeling [9–11]. However, few have evaluated these parameters in both systemic and synovial compartments, particularly in the context of trauma.

This study aims to fill this gap by characterizing the systemic and local immune profiles in patients with HA secondary to intra-articular fractures and comparing them with those in patients with isolated joint trauma and healthy individuals. A key goal was to identify immunological markers associated with more severe clinical courses, such as high effusion volume and recurrent aspiration, thereby laying the groundwork for immunologically guided stratification.

MATERIALS AND METHODS

Study Design and Setting

This was a prospective, comparative, clinical-laboratory study conducted from 2021 to 2024 at the Samarkand Branch of the Republican Specialized Scientific-Practical Medical Center of Traumatology and Orthopedics. Ethical approval was obtained from the Bukhara State Medical Institute's Local Ethics Committee, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Study Population

The study enrolled 150 individuals aged 18 to 70 years, divided into three groups: Main group (n=60): patients with acute hemarthrosis (HA) due to intra-articular fractures of the knee or ankle, confirmed by imaging (X-ray, CT, MRI) and, in some cases, arthroscopy; Comparative group (n=60): patients with isolated HA of the same joints, but without radiologically confirmed fractures; Control group (n=30): clinically healthy volunteers without musculoskeletal or systemic inflammatory disorders, age- and sex-matched to the clinical groups.

Inclusion criteria included acute traumatic joint injury (<48 hours), absence of autoimmune, infectious, or neoplastic diseases, and no prior immunosuppressive therapy. Patients with diabetes mellitus (decompensated), chronic kidney or liver disease, or active infection were excluded.

Clinical Evaluation

All patients underwent standardized clinical assessment including pain intensity (visual analogue scale), joint swelling, range of motion, and effusion volume. The need for puncture or surgical intervention (arthroscopy or osteosynthesis) was recorded, along with duration of hospitalization and incidence of early complications.

Immunological and Laboratory Methods

Systemic and synovial immune parameters were assessed at baseline (days 1–2), and in the main group at days 7, 14, and 30.

Cellular immunity: Peripheral lymphocyte subpopulations ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+/CD56^+$) were quantified via four-color flow cytometry (BD FACSCalibur, USA) using BD Simultest™ monoclonal antibody panels.

Humoral immunity: Serum levels of IgA, IgM, and IgG were measured via nephelometry (Siemens BN ProSpec, Germany). Circulating immune complexes (CIC) were quantified using polyethylene glycol precipitation. Complement components C3 and C4 were analyzed by radial immunodiffusion. Lysozyme was measured by turbidimetry using Micrococcus lysodeikticus assay.

Cytokines and MMP-9: Serum and synovial fluid concentrations of IL-1β, IL-6, TNF-α, IL-10, and MMP-9 were determined by sandwich ELISA using kits from

Vector-Best® (Russia), Cloud-Clone Corp.®, and R&D Systems® (USA).

Statistical Analysis

Data analysis was performed using Statistica 13.0 (StatSoft Inc.) and SPSS 26.0 (IBM). Distribution normality was assessed with the Shapiro–Wilk test. Quantitative data were presented as mean ± standard deviation (M ± SD) and compared using t-test or Mann–Whitney U test, as appropriate. Categorical variables were analyzed using χ^2 test.

Correlations between immune markers and clinical outcomes were assessed using Spearman's correlation coefficient. A logistic regression model was constructed to identify predictors of adverse outcomes. The model's accuracy was evaluated using ROC analysis, and the area under the curve (AUC) was reported. A p-value <0.05 was considered statistically significant.

RESULTS

Baseline Clinical Characteristics

A total of 150 individuals were included in the study: 60 in the main group (HA with intra-articular fractures), 60 in the comparative group (HA without fractures), and 30 healthy controls. The groups were comparable in terms of age and sex distribution (p>0.05). Mean hospitalization duration was significantly longer in the main group (14.2 ± 3.6 days vs. 9.8 ± 2.7 days; p<0.001), and the frequency of surgical interventions was higher (35% vs. 0%; p<0.001).

Cellular Immunity

Patients with hemarthrosis secondary to intra-articular fractures demonstrated a pronounced depression in cellular immunity:

Parameter	Control Group	Comparative Group	Main Group	p-value (Main vs. Comp.)
$CD3^+$ (×10⁹/L)	1.52 ± 0.30	1.21 ± 0.28*	1.03 ± 0.25*#	0.036
$CD4^+$ (×10⁹/L)	0.84 ± 0.19	0.71 ± 0.15*	0.59 ± 0.14*#	0.021
$CD8^+$ (×10⁹/L)	0.52 ± 0.11	0.46 ± 0.09	0.42 ± 0.08*#	0.042
$CD19^+$ (×10⁹/L)	0.21 ± 0.05	0.19 ± 0.04	0.17 ± 0.04*#	0.035
$CD16^+/CD56^+$ (NK)	0.28 ± 0.07	0.31 ± 0.06	0.38 ± 0.09*#	0.038

*Significantly different from control group (p<0.05);
#Significantly different from comparative group (p<0.05)

These findings indicate a trauma-induced suppression of both helper and cytotoxic T-cell compartments, especially in the presence of fractures, with a compensatory elevation of NK cell activity.

Humoral Immunity

Analysis of serum immunoglobulin levels and immune complexes showed significant alterations, particularly in the main group:

Parameter	Control	Comparative	Main Group	p-value
IgA (g/L)	2.41 ± 0.36	2.68 ± 0.42*	2.91 ± 0.45*#	0.031
IgM (g/L)	1.29 ± 0.25	1.12 ± 0.22*	0.98 ± 0.20*#	0.034
IgG (g/L)	11.2 ± 1.6	10.5 ± 1.5	9.4 ± 1.4*#	0.043
CIC (opt. units)	0.112 ± 0.018	0.138 ± 0.021*	0.165 ± 0.023*#	0.015
C3 (g/L)	1.12 ± 0.17	1.03 ± 0.15	0.89 ± 0.14*#	0.039
C4 (g/L)	0.32 ± 0.06	0.28 ± 0.05*	0.24 ± 0.05*#	0.027
Lysozyme (μg/mL)	1.75 ± 0.32	1.54 ± 0.30*	1.31 ± 0.28*#	0.022

Elevated IgA and CIC levels, along with reduced IgG, C3/C4, and lysozyme concentrations, suggest a shift toward chronic humoral activation with signs of immune exhaustion in the fracture group.

Cytokine Profiles

Proinflammatory cytokines were markedly increased in patients with intra-articular fractures, both systemically and locally:

Cytokine	Control (pg/mL)	Comparative	Main Group	p-value (Main vs. Comp.)
IL-6	8.8 ± 2.0	14.7 ± 3.1*	21.4 ± 3.5*#	0.019
IL-1β	6.4 ± 1.2	9.1 ± 1.6*	12.3 ± 2.1*#	0.028
TNF-α	7.2 ± 1.4	10.3 ± 2.0*	13.9 ± 2.2*#	0.034
IL-10	5.1 ± 0.9	6.7 ± 1.1*	8.2 ± 1.3*#	0.041

The elevation of IL-6 and TNF-α was particularly pronounced and correlated with effusion volume, pain scores, and delayed recovery.

Synovial Fluid Immunological Analysis

Analysis of synovial fluid samples obtained from patients with hemarthrosis in the knee joint revealed a robust local inflammatory response, particularly in the fracture group. Table summarizes comparative concentrations of key immune parameters in synovial fluid.

Immune Parameters in Synovial Fluid of the Knee Joint (M ± SD)

Parameter	Comparative Group	Main Group	p-value
Total leukocytes (×10⁶/L)	5.8 ± 1.4	8.9 ± 2.1	0.004
IL-1β (pg/mL)	42.1 ± 8.3	67.4 ± 10.2	<0.001
IL-6 (pg/mL)	58.7 ± 9.9	94.5 ± 12.8	<0.001
TNF-α (pg/mL)	36.4 ± 7.2	59.1 ± 8.7	0.002
IL-10 (pg/mL)	21.3 ± 4.1	28.6 ± 5.4	0.041
MMP-9 (ng/mL)	126.5 ± 18.4	214.8 ± 26.1	<0.001
IgG (g/L)	3.4 ± 0.9	5.1 ± 1.1	<0.01
CIC (optical units)	0.185 ± 0.03	0.266 ± 0.036	<0.01
Lysozyme (μg/mL)	1.12 ± 0.26	0.91 ± 0.23	<0.05

Patients with intra-articular fractures exhibited significantly higher synovial levels of proinflammatory cytokines (IL-1β, IL-6, TNF-α), immune complexes, and MMP-9. Notably, IL-6 and MMP-9 showed strong correlations with pain scores (VAS), joint swelling, and need

for repeated aspiration ($r = 0.71$ and 0.68, respectively; $p < 0.001$).

Conversely, lysozyme levels were significantly reduced in the fracture group, indicating local exhaustion of innate antimicrobial defense. IL-10 levels were also elevated in the fracture group, suggesting a compensatory counter-regulatory response to excessive inflammation.

DISCUSSION

This study revealed profound systemic and local immune disturbances in patients with posttraumatic hemarthrosis (HA) associated with intra-articular fractures of the lower limbs. Compared to isolated joint trauma without fracture, the immunological profile of these patients was characterized by a combination of adaptive immune suppression, humoral dysregulation, and exaggerated cytokine-mediated inflammation.

Cellular Immune Suppression:

A significant reduction in $CD4^+$ and $CD8^+$ T lymphocytes was observed in the fracture group, indicating a posttraumatic secondary immunodeficiency. This is consistent with reports that trauma induces lymphocyte apoptosis and redistribution, impairing T cell-dependent immune surveillance [1,2]. The decreased CD4/CD8 ratio observed in our patients mirrors findings in septic and polytraumatized individuals, suggesting a similar immunoregulatory failure [3].

Compensatory Innate Immune Activation:

A paradoxical increase in NK cells and CIC was noted in the same patients, reflecting a compensatory shift toward innate immunity. This may serve to contain early tissue damage but can also amplify inflammatory cascades. Our findings align with studies demonstrating that NK cell expansion post-injury is associated with both protective and deleterious effects depending on the regulatory balance [4].

Humoral Dysregulation and Immune Complex Accumulation:

The fracture group showed increased serum IgA and CIC but decreased IgM and IgG levels, consistent with immune exhaustion. The elevation in CIC suggests antigen–antibody complex deposition, a known driver of chronic synovitis and tissue injury [5,6]. Simultaneous consumption of complement components (C3, C4) supports this mechanism.

Synovial Cytokine Storm:

Cytokine analysis of synovial fluid revealed a pronounced proinflammatory signature, with IL-6, IL-1β, and TNF-α significantly elevated in fracture patients. These cytokines are known to induce chondrocyte apoptosis, upregulate matrix-degrading enzymes, and promote angiogenesis—hallmarks of chronic joint damage [7,8]. High synovial IL-6 correlated with clinical severity, consistent with its role as a biomarker of joint pathology [9].

MMP-9 as a Predictor of Joint Destruction:

Elevated MMP-9 levels in the synovial fluid of fracture patients were strongly associated with repeated effusions and prolonged functional impairment. This enzyme degrades extracellular matrix and basement membranes, facilitating leukocyte infiltration and irreversible joint damage [10]. Similar results have been reported in both rheumatoid arthritis and severe posttraumatic arthritis models [11].

Regulatory Failure:

Although IL-10 levels were increased in both serum and synovial fluid, the IL-6/IL-10 ratio remained high in fracture patients, suggesting insufficient anti-inflammatory counterbalance. This imbalance may contribute to unresolved inflammation and subclinical joint remodeling [12].

Taken together, these findings support the concept that intra-articular fractures not only cause mechanical damage but also provoke a sustained immunopathological process. The identified immunological markers—particularly IL-6, MMP-9, $CD4^+$ T cells, CIC, and lysozyme—may serve as candidate indicators for prognosis and therapeutic targeting. Future studies should explore whether immune-guided stratification can inform personalized management protocols.

CONCLUSION

Intra-articular fractures complicated by hemarthrosis are not solely mechanical injuries but involve a distinct and measurable immunoinflammatory cascade. Our study demonstrates that these patients exhibit: Profound depression of T cell immunity (notably $CD4^+$); Compensatory activation of NK cells and innate humoral mechanisms; Increased formation of circulating immune complexes and complement consumption; Elevated levels of proinflammatory cytokines (IL-6, TNF-α, IL-1β) both systemically and intra-articularly; Significant upregulation of synovial matrix metalloproteinase-9 (MMP-9), associated with worse clinical outcomes.

These findings collectively underscore the immunological complexity of posttraumatic hemarthrosis and support the inclusion of immunological assessment in early clinical evaluation. Markers such as IL-6, $CD4^+$

count, CIC, MMP-9, and lysozyme may aid in risk stratification and guide personalized treatment strategies.

Future directions should include validation of prognostic immune panels and investigation of targeted immunomodulatory interventions in posttraumatic joint care.

Ethical Approval:

This study was approved by the Local Ethics Committee of Bukhara State Medical Institute. All participants provided written informed consent in accordance with the Declaration of Helsinki (2013 revision).

Conflict of Interest:

The authors declare no conflicts of interest.

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Author Contributions:

Khamdamov B.Z.: Conceptualization, methodology, supervision, final review.

Vakhabov K.Sh.: Data collection, statistical analysis, manuscript writing.

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TRAVMADAN KEYINGI GEMARTROZ BILAN KECHUVCHI ICHKI BO‘G‘IM SINISHLARIDA MAHALLIY YALLIG‘LANISH VA IMMUNOLOGIK KO‘RSATKICHLAR PROFILI

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ANNOTATSIYA:

Ichki bo‘g‘im sinishlari bilan bog‘liq travmatik gemartroz holatlarida yallig‘lanish jarayonining mahalliy va tizimli immunologik mexanizmlarini baholash ushbu tadqiqotning asosiy maqsadini tashkil etdi. 150 nafar bemor ishtirokida o‘tkazilgan ushbu prospektiv klinik-laborator tadqiqotda T-limfotsitlar subpopulyatsiyasi, immunoglobulinlar, tsitokinlar (IL-6, TNF-α, IL-1β, IL-10), matritsali metalloproteinaza-9 (MMP-9) va lizosim kabi ko‘rsatkichlarning qon zardobi va sinovial suyuqlikdagi darajalari aniqlangan. Olingan natijalar travmadan keyingi yallig‘lanish intensivligi va klinik oqibatlar o‘rtasidagi yaqqol bog‘liqlikni ko‘rsatdi. IL-6, MMP-9, $CD4^+$ va tsirkulyatsiyalovchi immun komplekslar asosiy prognoz ko‘rsatkichlari sifatida ajratib ko‘rsatildi.

Kalit so‘zlar: Gemartroz, ichki bo‘g‘im sinishi, immunologiya, tsitokinlar, MMP-9, sinovial suyuqlik, yallig‘lanish, prognoz.

ИММУНОЛОГИЧЕСКИЙ ПРОФИЛЬ И ЛОКАЛЬНЫЕ МЕХАНИЗМЫ ВОСПАЛЕНИЯ ПРИ ПОСТТРАВМАТИЧЕСКОМ ГЕМАРТРОЗЕ, ВОЗНИКАЮЩЕМ НА ФОНЕ ВНУТРИСУСТАВНЫХ ПЕРЕЛОМОВ НИЖНИХ КОНЕЧНОСТЕЙ

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АННОТАЦИЯ:

Целью настоящего исследования явилась оценка особенностей системного и локального иммунного ответа у пациентов с гемартрозом, возникающим при внутрисуставных переломах нижних конечностей. В рамках проспективного клинико-лабораторного анализа, проведённого на 150 пациентах, определяли уровни субпопуляций лимфоцитов, иммуноглобулинов, циркулирующих иммунных комплексов, цитокинов (IL-6, IL-1β, TNF-α, IL-10), матриксной металлопротеиназы-9 (ММП-9) и лизоцима в сыворотке крови и синовиальной жидкости. Результаты показали выраженную активацию воспалительного каскада при наличии внутрисуставного перелома, а также достоверную корреляцию иммунологических параметров с объёмом излияния, болевым синдромом и необходимостью повторных вмешательств. Ключевыми прогностическими маркерами выступили IL-6, ММП-9, $CD4^+$ и циркулирующие иммунные комплексы.

Ключевые слова: Гемартроз, внутрисуставной перелом, иммунитет, цитокины, ММП-9, синовиальная жидкость, воспаление, прогноз.