Systemic Immune Profiling to Predict Infection in Patients with Bone Injuries

Kelly E Leguineche[1], Jena L Robinson[2], Lauren C Hill[2], Danielle Olson[3], Raj Karia[3], Samantha Nino[3,4], Molly M Moore[3,4], Anastasiyah Bahdanovich[2], Auveen Hajarizadeh*[1], Kimberly A Jones[1], Roman M Natoli[2], Philipp Leucht[3], Todd O McKinley[3], and Robert E Guldberg[1]
[1]University of Oregon, Eugene, OR, [2]Indiana Methodist Hospital, Indianapolis IN, [3]NYU Langone Medical Center, New York NY, [4]Eskenazi Health Medical Center, Indianapolis IN
auveenh@uoregon.edu

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INTRODUCTION: Despite bone's inherent regenerative capacity, severe extremity trauma often results in high complication rates and poor functional recovery. Fracture related infection impacts nearly 42% of open fracture patients and remains one of the leading causes of amputation. Early and accurate diagnosis of fracture-related infection is therefore critical for improving outcomes, minimizing tissue damage, and increasing the likelihood of limb salvage. However, current diagnostic approaches are limited—overt clinical signs of infection often appear only 6–12 weeks post-operatively, delaying intervention and complicating recovery. Clinically used systemic biomarkers such as C-reactive protein (CRP), white blood cell count (WBC), and erythrocyte sedimentation rate (ESR) offer some diagnostic value but lack the specificity and accuracy (52–80%) required for confident clinical decision-making. More invasive diagnostic procedures, including wound swabs or tissue biopsies, can further burden patients and delay treatment. Consequently, there is an urgent need for reliable, noninvasive tools that can detect infection at earlier stages. Previous work in preclinical animal models has shown that local infections disrupt systemic cell and cytokine profiles, increasing immunosuppressive myeloid-derived suppressor cells (MDSCs) and reducing immune effector cells like T cells in peripheral blood. Yet, these immune alterations have not been fully translated into clinically actionable diagnostic strategies. Because the immune system's balance depends on the precise coordination of diverse cellular and molecular components, characterizing these changes could reveal novel biomarkers of infection. To address this gap, our study analyzes blood from fracture patients and radiographically healed patients using advanced machine learning methods to identify immune signatures predictive of infection risk and healing outcomes.
METHODS: A cohort of 11 patients (6 male, 5 female; ages 23–66) presenting with Type I–IIIA open tibia fractures were identified as either radiographically healed or having developed a surgical site infection. Peripheral blood samples were collected at multiple clinical timepoints, including pre-operation (Draw 1), point of discharge (Draw 2), and follow-up visits at approximately 2–4 weeks (Draw 3), 6–8 weeks (Draw 4), and 12–14 weeks (Draw 5). For cellular profiling, whole blood was collected into K_2EDTA tubes, diluted 1:9 in cryopreservation medium (CryoStor CS10), and stored at −80 °C until analysis. Upon thawing, samples underwent red blood cell lysis, and a small aliquot was used to assess viability and cell counts using the NC-200 Cell Viability Via2 assay. The remaining cells were stained with a fixable amine-reactive viability dye (Zombie UV, BioLegend), fixed, and subsequently labeled with a 15-antibody panel designed to quantify key immune cell subsets. Samples were acquired on an Aurora Cytek spectral flow cytometer, and data were analyzed using FlowJo for gating and quantification of cell populations. Downstream statistical and computational analyses were conducted in R, utilizing the tidyverse, edgeR, and mixOmics packages for data preprocessing, differential abundance testing, and multivariate modeling.
RESULTS: We quantified 16 immune cell frequencies across two timepoints—Draw 1 (D1) and Draw 3 (D3). Compared to patients who healed successfully, those who developed surgical site infections exhibited distinct shifts in circulating immune populations. Plasmacytoid dendritic cells (pDCs) were significantly elevated in infected patients at D3, while regulatory T cells (Tregs) showed a moderate but significant increase, suggesting a shift toward an immunosuppressive phenotype during infection. In contrast, myeloid cell frequencies tended to decrease in infected individuals, consistent with impaired innate immune activation (Figure 1A). We next performed sparse partial least squares discriminant analysis (sPLS-DA) to identify whether early immune cell profiles could accurately classify infected from healed patients. The first two components accounted for 17.5% and 13.0% of the total variance, respectively. Infected and healed patients separated along the component 1 axis (Figure 1B). The top five variables in Component 1 included D3 pDCs, D3 Tregs, D1 T cells, D1 pDCs, and D1 myeloid cells—supporting the observation that infection is associated with early immune dysregulation across innate and adaptive compartments (Figure 1C).
DISCUSSION: Our approach identified distinct systemic immune alterations in circulating immune cell composition that distinguish patients who develop surgical site infection from those who heal successfully. The sPLS-DA model revealed that both early and late immune signatures—particularly changes in pDCs, Tregs, and myeloid populations—contribute to outcome differentiation, suggesting that infection is not merely a late complication but a continuum of immune dysregulation that may begin prior to overt clinical symptoms. Detecting these alterations in peripheral blood underscores the utility of this methodology for noninvasive immune monitoring and highlights its potential to uncover biomarkers predictive of infection risk. While limited by small sample size, these findings validate the technical approach and support its future application in larger cohorts to refine predictive modeling and integrate immune profiling into clinical decision-making for orthopedic trauma.
SIGNIFICANCE/CLINICAL RELEVANCE: This work addresses a critical barrier in orthopedic trauma care by advancing early, noninvasive detection of fracture-related infection. Successful validation of immune-based biomarkers could transform clinical decision-making by enabling earlier intervention, improving limb salvage rates, and reducing long-term morbidity in patients with severe extremity injuries.

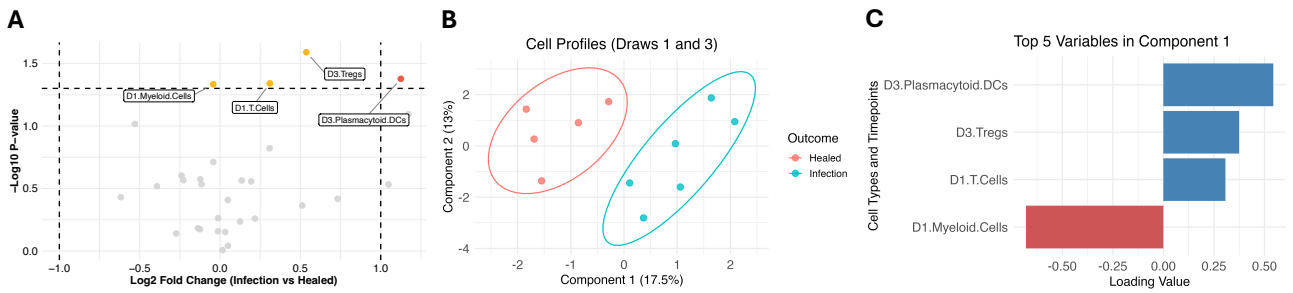

Figure 1. Immune profiling reveals systemic changes linked to surgical site infection after open tibia fracture. **(A)** Volcano plot showing differences in circulating immune cell populations between infected and healed patients; dashed lines mark $\log_2$ fold change (±1) and $-\log_{10}$ p-value (~1.3) thresholds. **(B)** sPLS-DA plot separates infection and healed groups along Component 1 (17.5% variance) driven by immune composition. **(C)** Top five contributors to Component 1, with late pDCs and Tregs showing strong positive loadings and early myeloid cells negative loadings.