Using the Plasma Proteome to Define the Temporal Progression of Normal and Nonunion Human Humerus Fracture Healing

Nazanin Nafisi, MS, Serkalem Demissie, PhD, Avani Vaishnav, MD, Ryan Kim, MD, Jason Lowe; MD, Robert O'Toole; MD, David B. Weiss; MD, Paul Matusewski, MD, George Hanson, MD, Joseph T. Patterson MD; MD, Roman M. Natoli, MD PhD; Suna Chung, MPH , Renan Castillo PhD , Paul Tornetta III MD, Louis Gerstenfeld PhD on behalf of the METRC Major Extremity Trauma Research Consortium

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Introduction: Fracture healing remains a central challenge in musculoskeletal medicine, with clinical assessments typically relying on radiography and patient-reported outcomes that offer little insight into the underlying biology. The inability to detect delayed or failed healing (nonunion) in its early stages severely limits both timely intervention and mechanistic research. Recent advances in plasma proteomics present an opportunity to profile molecular events during bone repair and to distinguish successful from aberrant healing in patients.

Methods: We conducted a prospective, multicenter analysis of the plasma proteome in 18-70 year old adults with isolated, closed, non-articular humeral fractures (AO/OTA 11 type A2.1, A2.2 and/or A2.3) with diaphysis involvement or a diagnosis of isolated closed extra-articular fractures of the humeral shaft (AO/OTA 12 A, B, or C). All were managed non-operatively. A total of 32 patients, 10 males and 22 females were enrolled with 24 union and 7 nonunion completing the study. Serial plasma samples, radiological and PROMIS data for pain and function were obtained at 5 standardized time points over a six month period. Proteomic profiling was performed using the SomaLogic platform, quantifying approximately 7,000 proteins per sample. Clinical progression was tracked using the modified Radiographic Union Score for Tibia (mRUST) and PROMIS patient-reported outcome measures. Nonunion assessed at the 7-9 week recall period and was based on both an mRUST score of less than 6 and motion at the site while definitive healing was determined based on a mRUST score of 12 or greater and no motion. Statistical analysis determined differentially expressed proteins (DEPs) through mixed-effects regression and had to have log2 fold-change $\geq$1.3, FDR $\leq$0.05 difference from either the first or last time point sample. Biological pathway analysis utilized GO, KEGG, and IPA resources. Proteomic profiles were compared temporally for the first three recall time (up to 9 weeks) comparing the union and nonunion groups. Only proteins that appeared both in the normal healing DEP group and which showed $p \leq 0.05$ at one of the three first recall times when comparing union and nonunion groups were considered as significant determinates for nonunion. This study was carried out under a uniform single IRB protocol administered by Major Extremity Trauma Consortium (METRC).

Results: Patients determined as nonunion at 7-9 weeks had lower mRUST scores at every recall time even prior to 7-9 weeks. In contrast, patients determined as progressing in their healing all increased with time (Figure A). PROMIS pain interference and intensity scores were inversely correlated with both radiological indices and decreased over time while always being greater in nonunion patients. Proteomic analysis identified 118 plasma proteins with significant temporal changes during normal healing (log2 fold-change $\geq$1.3, FDR $\leq$0.05), with a subset showing strong upregulation for processes related to endochondral bone formation and extracellular matrix remodeling in the first 12 to 16 weeks after injury. Four common cartilage ECM proteins that were in our DEP group are presented (Figure B). Markers of inflammation and the acute phase response peaked early post-injury but were subsequently suppressed as healing advanced. Patients who progressed to nonunion demonstrated a distinct proteomic trajectory: 41 plasma proteins were differentially expressed relative to union patients during the first nine weeks, with ten of these proteins also found in the normal healing DEP group. The five proteins associated with ECM formation in this group are shown in Figure 3C. Nonunion was characterized biologically by failed upregulation of specific extracellular matrix proteins and prolonged suppression of key acute phase proteins. Importantly, these differences were detectable as early as one to three weeks after injury, preceding the identification of nonunion by radiological diagnosis.

Discussion and Conclusion: These findings demonstrate that high-dimensional plasma proteomic profiling can temporally resolve the sequence of biological events underlying human fracture healing. The plasma proteome reflects both local bone and systemic responses, capturing key phases from early inflammation through cartilage and bone formation. The differences in plasma protein signatures between union and nonunion patients precede currently available clinical indicators, suggesting that plasma markers can be developed for early diagnosis and potential intervention. The nature of the nonunion DEP suggests that nonunion is associated with an altered acute phase response and is selective for ECM adhesion or ECM matrix assembly but are not structural ECM proteins.

Significance: This is the first comprehensive study to map the temporal plasma proteome throughout human fracture healing and nonunion. Our results identify novel, actionable biomarkers that could potentially be developed to predict failed healing within weeks of injury—well before conventional methods allow for diagnosis. This study lays the groundwork for the development of biomarker-driven precision diagnostics that could be used in the assessment of therapies for in orthopedic trauma and provides a molecular blueprint for translating basic science insights into clinical care.

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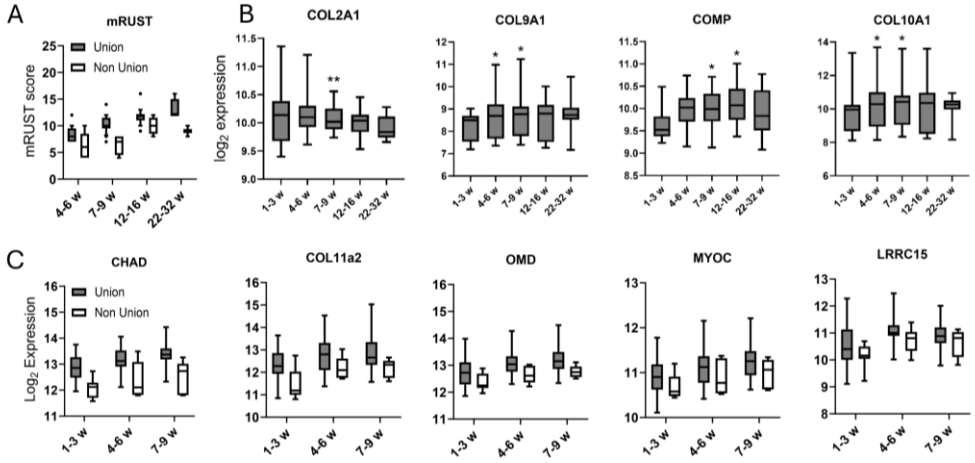

Figure 1 A Comparisons of mRUST for Union and Nonunion Patients. * those time points showing difference in level **B)** Relative Expression Values four cartilage ECM proteins found in the normal healing DEP. * those time points showing difference relative to first or ** last assay time point. **C)** Expression Values of five proteins associated with ECM found comparing union to nonunion.