Adult Collagen X Fracture Biomarker Levels Demonstrate Early Peak After Fracture to Match Preclinical Model

***Zachary Mark Working, MD**; Kaitlyn E. Whitney, BS; Justin Hellwinkel, MD; Lauren Pierpoint PhD; Ryan Coghlan MS; Darin M. Friess MD; Theodore Miclau, MD; William Horton, MD; Brian Johnstone, PhD; Chelsea S. Bahney, PhD*
OHSU, UCSF, SPRI, Portland, OR, United States

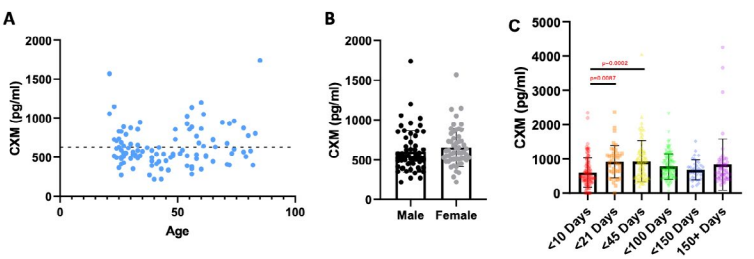
Purpose: There exists no validated method to quantify biologic fracture healing. Many fractures heal via callus and a cartilage intermediate. Collagen X (ColX) is expressed during cartilage-to-bone transformation, providing a diagnostic opportunity. A published preclinical murine fracture model (novel validated sandwich ELISA [enzyme-linked immunosorbent assay]-based assay: "CXM") demonstrates early gene expression (day 7) after fracture, a delayed serum CXM peak (day 14), and supportive immunohistochemistry staining (ColX). Here we present preliminary CXM data from an adult fracture observational cohort.

Methods: After IRB approval, patients presenting within 14 days (isolated fracture) were approached. Dried blood spots (DBS) were collected from lancet/fingerprick via protein cards (injury/2/6/12 weeks, all other visits). DBS sampling involved 3.1-mm punch in duplicate, +250 mL of sample diluent, and overnight extraction (4C) before assay. Fracture patients required 3 samples for analysis. We expected a sharp early peak followed by resolution to match our preclinical model. Healthy volunteers were also assayed.

Results: Healthy controls: 113 patients; no difference between baseline CXM in men and women (603.3 pg/mL, n = 59 vs 653.5 pg/mL, n = 54; $P = 0.29$) nor by age ($P = 0.24$, r = 0.11). Adult fracture cohort: 110 of 160 produced 3 sequential samples for analysis (424 samples, median days from fracture = 43, maximum 597, median age 48.5 years [range, 19.7-83.2]; 60 of 110 [54.5%] male). Samples were binned by days from fracture: 0-9, 10-20, 21-44, 45-99, 100-149, and 150+. There was a significant difference between samples at 10 to 20 days ($P = 0.0087$) and samples at 21 to 44 days ($P = 0.0002$) compared to 0 to 9 days, signifying an early CXM spike post-fracture.

Conclusion: CXM levels in humans demonstrate an early peak post-fracture, matching published preclinical findings, providing an opportunity to develop a biomarker for adult fracture care. Future efforts remain focused on cohort expansion to account for broad heterogeneity in an adult fracture population.

FIGURE 1: CXM LEVELS IN ADULTS



CXM in healthy adults: (A) No correlation between age and CXM levels (r=0.112, p=0.241). (B) No statistically significant difference between CXM levels between males and females (p=0.287).
CXM in adult fracture patients: (C) Significant differences found in CXM magnitude between samples from 0-9 days after fracture to both 10-20 day samples and 21-44 day samples, illustrating expected spike in CXM response.