Bioassay of Circulating Collagen X Degradation Product Correlates with Mouse and Human Fracture Healing Progression

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Purpose: No tool exists to quantitatively measure fracture healing. The purpose of this study is to validate a bioassay for collagen X (CXM, intact trimeric noncollagenous-1 domain) as a marker of endochondral ossification to quantitatively assess fracture biology in a robust murine model and preliminary human testing.

Methods: Closed midshaft tibia fractures were created using a classic drop-weight device. Serum was collected from mice at euthanasia at 5, 7, 10, 14, 21, 28, and 42 days postinjury and compared to uninjured controls. Fractured tibias were harvested for gene/histology analysis. Separately, 32 healthy and 16 injured (long bones) humans consented for fingerprick blood draw for CXM analysis.

Results: Murine data supports peak and resolution of CXM after fracture: Serum collected from mice after euthanasia shows a CXM peak (14 days). CXM values change over time in both male ($F < 0.001$) and female ($F < 0.001$) mice. Male CXM at day 14 is significantly higher than at all other time points (Tukey's honestly significant difference (HSD), $P < 0.01$, $P < 0.05$ at day 7). Similarly, female CXM at day 14 was significantly increased compared to days 0, 5, and 21 postfracture ($P < 0.01$). No significant difference in baseline CXM was found between uninjured adult males and females. Human CXM corresponds to fracture progression: Healthy adult baseline CXM established at 71.8-629.6 ng/mL, median of 253.3 ng/mL, without clear age/sex-related trends (age 20-63 years). Long bone fracture healing (n = 16) shows a CXM peak between 10 and 20 days postfracture. Elderly patients (>60 years) demonstrate elongated CXM peak corresponding with clinical and preclinical evidence for delayed healing.

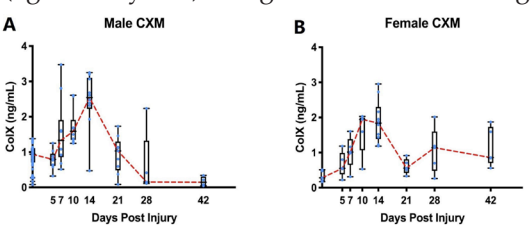

Fig. 1A-B Serum collected from mice following euthanasia between 5 and 42 days post-fracture shows a peak in CXM at 14 days post-fracture. CXM values change over time in both male ($F_{7,81} < 0.001$) and female ($F_{7,32} < 0.001$) mice. Tukey's HSD indicates that male CXM at day 14 is statistically higher than at all other time points ($p < 0.01$, $p < 0.05$ at Day 7). Similarly in female mice, CXM at day 14 was significantly increased compared to days 0, 5, and 21 post-fracture ($p < 0.01$).

Conclusion: This proof of concept study via murine model and limited human subjects suggests collagen X may be a reasonable first quantifiable biomarker of fracture healing. Human trials have begun.

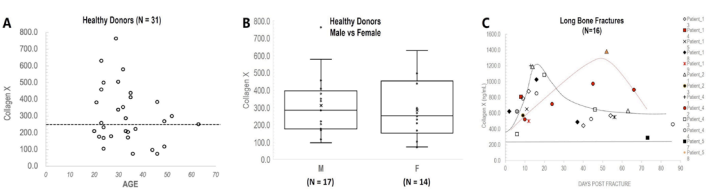

Fig. 2 Human CXM corresponds to fracture progression. (**A-B**) Healthy adults show a range of 71.8-629.6 ng/ml and a median value of 253.3 ng/ml (grey line), and no clear age- or sex-related trends associated with CXM levels in a cohort ranging from 20-63 years old. (**C**) Long bone fracture healing from an initial cohort of 16 patients shows a similar peak in CXM levels between 10-20 days post fracture (black dotted trend lines). In patients over 60 years old, we see a shifted CXM peak that corresponds with clinical and preclinical evidence for delayed healing in elderly patients (red dotted trend lines).

See the meeting app for complete listing of authors' disclosure information.