

### Evaluation of Serum Bone Markers for Early Diagnosis of Non-union and Infected Non-union

**Summary:** The objective of this pilot study was to evaluate the use of serum bone markers for early diagnosis of infection and non-union in fractures. The researchers discovered that, in a rabbit non-union and infected non-union model, a combination of bone markers was useful for predicting infection at 4 weeks.

Non-union and infected non-union are devastating complications of fracture repair in horses. Early diagnosis and treatment is essential for a favorable outcome. Currently available diagnostic techniques have limitations for evaluation of healing following fracture repair in horses. Novel methods for early diagnosis of non-union and infected non-union are needed. Serum markers of bone formation and resorption have been shown to change following long-bone fracture. There have been no controlled studies evaluating the use of serum bone markers for early diagnosis of infection and non-union.

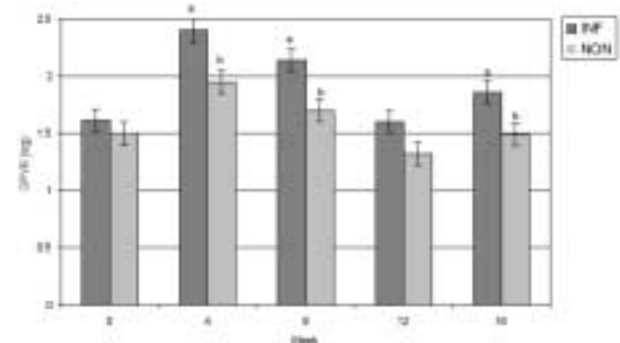
The objective of this study was to evaluate the use of serum bone markers for early diagnosis of infection and non-union. This led to the hypothesis that animals with infection and non-union will have different concentrations of serum bone markers compared to animals with non-infected fractures and normal union. A rabbit non-union and infected non-union model was used because this was a pilot study. All procedures were approved by the Animal Care and Use Committee. This research was in partial fulfillment of a PhD for Dr. Louise Southwood. Drs. David Frisbie, Chris Kawcak, and Wayne McIlwraith were integral in study design and interpretation of the results.

Thirty-two skeletally mature New Zealand White rabbits were used to evaluate the use of deoxypyridinoline crosslinks for early diagnosis of infection and non-union. This study was performed as part of another study evaluating the use of adenoviral transfer of the bone morphogenetic-2 gene (Ad-BMP-2) for enhancement of healing in infected non-unions. A femoral fracture defect with plate and screw fixation was used as a non-union model. Animals were assigned to one of four groups: Ad-Luciferase (Luc) control (non-union), Ad-BMP-2 treated (normal union), Infected Ad-Luc (infected non-union) and Infected Ad-BMP-2 (unknown). Radiographic bone lysis and callus formation was graded from 0 to 4 (0=none to 4=marked). Blood was collected preoperatively (0) and at 4, 8, 12, and 16 weeks postoperatively. Osteocalcin (OC) and bone-specific alkaline phosphatase (BS-ALP) were measured as markers of bone formation. Deoxypyridinoline

crosslinks (DPYR) were measured as a marker of bone resorption. The serum concentration of OC, BS-ALP, and DPYR were measured using a commercially available kit (Quidel Corporation, Mountain View, CA). Data was analyzed using a mixed-model analysis of variance and Pearson's correlation. The level of significance was  $p < 0.05$ .

Serum bone marker concentration changed over time. Markers of bone formation (OC, BS-ALP) decreased from time 0 at 4 weeks, peaked at week 8 and then decreased, whereas the marker of bone degradation (DPYR) peaked at week 4 and then decreased. Both OC and BS-ALP were lower in infected rabbits at 4 weeks, and OC was higher in infected rabbits at 16 weeks, whereas DPYR was higher in infected rabbits at 4, 8, 12, and 16 weeks (Figure 1). A combination of bone markers was useful for predicting infection at 4 weeks, with an accuracy of 96%. There were correlations between lysis grade and BS-ALP and DPYR at 4 weeks. There were only weak associations between callus grade and serum bone marker concentrations.

Future studies in the use of serum bone marker concentration will be performed in the horse for evaluation of fracture healing and infection.



**Figure 1.** A plot illustrating the association between the concentration of deoxypyridinoline crosslinks (DPYR) and infection, over time. The sample at time 0 was collected preoperatively and the other samples 4, 8, 12, and 16 weeks after surgery. Data are expressed as least squared means  $\pm$  standard error of the mean. Different letters represent statistically significant differences. INF=infected, NON=non-infected.

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