

Evaluation of Nuclear Scintigraphy for Early Diagnosis of Non-union and Infected Non-union

Summary: The objective of this study was to evaluate the use of Tc-PO (technetium-labeled diphosphonate) and Tc-CIPRO (technetium-labeled ciprofloxacin) for early diagnosis of non-union and infection. The results showed that technetium-labeled diphosphonate and ciprofloxacin may be useful for diagnosis of infected fractures late in fracture healing. There were a high number of false positive and false negative results early in fracture healing, probably as a result of increase in blood flow to the fracture site as well as an increase in bone metabolism. Evaluation of uptake ratios over time and the distribution of uptake along the affected bone may be more valuable than a single ratio. Future studies in the target species (equine) are required.

Non-union and infected non-union are devastating complications following fracture repair in horses. Early diagnosis and treatment is essential for a favorable outcome. Currently available diagnostic techniques have limited use for evaluation of equine fracture repair. Technetium-labeled diphosphonate (Tc-PO₄) is used to evaluate bone metabolism. Technetium-labeled ciprofloxacin (Tc-CIPRO) is used to evaluate the presence of infection. There have been no controlled animal studies evaluating the use of Tc-PO for diagnosis of an infected non-union. Therefore the objective of this study was to evaluate the use of Tc-PO and Tc-CIPRO for early diagnosis of non-union and infection. This led to the hypothesis that animals with non-union and infection will have different scintigraphy findings compared to animals with normal union and non-infected fractures. 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.

The project was for partial fulfillment of a PhD for Dr. Louise Southwood. Drs Kawcak, Frisbie, and McIlwraith were fundamental in the study design, analysis, and data interpretation. Dr Philip Steyn is a Board Certified Radiologist who specializes in nuclear scintigraphy, he performed the subjective analysis, and was integral in the designing the scanning methods as well as data analysis.

Thirty-two skeletally mature New Zealand White rabbits were used. 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. Rabbits 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). Nuclear scintigraphy was performed 4, 8, 12, and 16 weeks postoperatively; Tc-PO scanning was performed initially and Tc-CIPRO was performed 48-hours later. Lateral and craniocaudal views were used. Ratios of nucleotide uptake between the operated and normal limbs were determined for a specific view and region of interest (ROI), which included the femoral defect and whole bone. Data were analyzed using a mixed-model analysis of variance. Level of significance was $p < 0.05$.

There was a difference in uptake ratios between infected and non-infected animals with Tc-PO at 8, 12, and 16 weeks; at 4 weeks, there was no difference between infected and non-infected animals. Although Tc-CIPRO was better than Tc-PO at identifying the presence of infection, the accuracy was variable at 4 weeks depending on the view and the ROI (Figure 1). There was a good correlation between radiographic lysis grade and uptake ratio after 4 weeks ($r^2=0.7$) for both Tc-PO and Tc-CIPRO. When Tc-PO was used to predict whether animals were infected or not infected, the accuracy was good after 4 weeks (>80%). Similarly, when Tc-CIPRO was used to predict infection, the accuracy was as low as 61% at 4 weeks. However, it was up to 100% after 4 weeks, depending on the view and ROI. When scintigraphy findings using Tc-99m-ciprofloxacin were evaluated by a board certified radiologist for infected versus not infected, there was a high incidence of false positives (low specificity). Animals that developed a large bridging callus (grade 4) had the highest ratios at 4, 8, and 12 weeks then the ratios declined relative to the other animals at 16 weeks, whereas animals that had callus formation but had not healed the defect (grade 2 and 3), the ratio continued to increase. Animals with defects that were not healing (grade 1) had a low uptake ratio that did not increase with time (Figure 2).

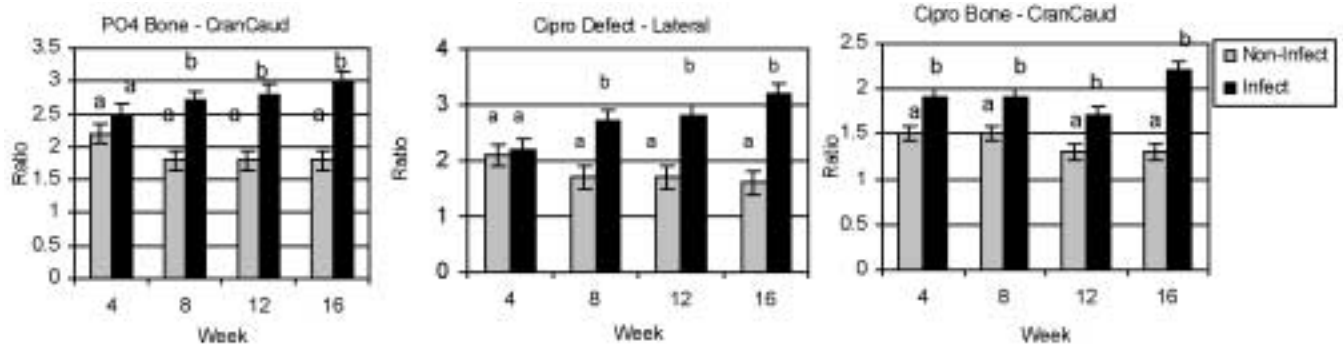


Figure 1. Plots showing the association between radionuclide uptake ratio and infection. The radionuclide uptake ratio is on the y-axis and the week postoperative is on the x-axis. There was a significant difference between infected and non-infected rabbits at 8, 12, and 16 weeks for both the Tc-PO4 and -CIPRO scans. At 4 weeks the ability of both Tc-PO4 and -CIPRO scans to distinguish infected from non-infected fractures was dependent on the view and the region of interest (ROI). The craniocaudal view and the entire bone were the best view and ROI for evaluating infection, whereas using the lateral view and defect ROI, there was no difference between infected and non-infected fractures at 4 weeks. Data are presented as least squared means (LSM) +/- standard error (SE); different letters represent statistically significant differences.

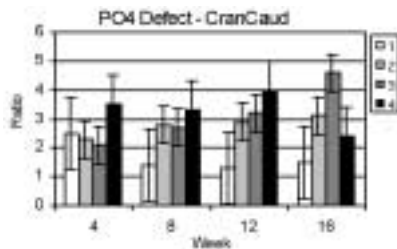


Figure 2. A plot showing the association between radionuclide (Tc-PO4) uptake ratio and callus formation. Uptake ratio is shown on the y-axis and week postoperative on the x-axis. There was a trend for rabbits with higher callus grade to have a higher uptake ratio at 8 and 12 weeks. Rabbits that healed the defect (grade 4) then decreased after week 12. Data are presented as least squared means (LSM) +/- standard error (SE).

Technetium-labeled diphosphonate and ciprofloxacin may be useful for diagnosis of infected fractures late in fracture healing. There were a high number of false positive and false negative results early in fracture healing, probably as a result of increase in blood flow to the fracture site as well as an increase in bone metabolism. Evaluation of uptake ratios over time and the distribution of uptake along the affected bone may be more valuable than a single ratio. Future studies in the target species (equine) are required.

Acknowledgements: This project was funded by Morris Animal Foundation.