SERUM BONE MARKERS FOR ASSESSMENT OF FRACTURE HEALING AND EARLY DIAGNOSIS OF OSTEOMYELITIS IN A RABBIT NON-UNION MODEL

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Introduction: Non-union and infected non-union are devastating clinical complications following fracture repair. Early diagnosis is required for prompt treatment intervention, which is essential for a favorable outcome. Imaging modalities that are available for assessing fracture healing have limitations, particularly when metallic implants are used. The concentration of serum and urine bone markers has been shown to change with various bone diseases, including fracture and fracture healing. Although there have been clinical studies evaluating changes in bone marker concentration following fracture and during fracture healing, there have been no controlled experimental studies evaluating the use of serum bone markers for early diagnosis of non-union and osteomyelitis. The objective of this study was to evaluate the use of the serum bone markers, osteocalcin (OC), bone-specific alkaline phosphatase (BS-ALP), and deoxypyridinoline crosslinks (DPYR) for assessing fracture healing and early diagnosis of osteomyelitis using a non-union and infected non-union rabbit model. The study was designed to test the hypothesis that markers of bone formation (OC and BS-ALP) will be higher in rabbits that healed compared to rabbits that developed a non-union, and that a marker of bone resorption (DPYR) will be higher in infected compared to non-infected rabbits.

Methods: Thirty-two skeletally mature New Zealand White rabbits with a unilateral femoral defect stabilized with plates and screws were used. This study was part of a larger study to evaluate adenoviral transfer of bone morphogenetic-2 gene (Ad-BMP-2) for enhancing fracture healing in an infected non-union model. Experimental groups were: (1) non-union Ad-Luciferase (Ad-LUC) control (NON-LUC), (2) non-union Ad-BMP-2 treated (NON-BMP), (3) infected non-union Ad-LUC control (INF-LUC), and (4) infected non-union Ad-BMP-2 treated (INF-BMP). Serum was collected preoperatively, and 4, 8, 12, and 16 weeks postoperatively. Rabbits in the infected groups were inoculated in the defect with Staphylococcus aureus. Quantitative aerobic culture was performed on all rabbits following euthanasia to confirm the presence or absence of infection. Serum was analyzed for OC, BS-ALP, and DPYR using commercially available kits (Quidel Corporation, Santa Clara, CA). Radiographic callus and lysis grades at 16 weeks were used as clinical outcome measurements for fracture healing and osteomyelitis, respectively. Data were normalized using a log-transformation and analyzed using an ANOVA. A p-value <0.05 was considered statistically significant. All procedures were approved by the Colorado State University Animal Care and Use Committee.

Results: There was a significant association between serum bone marker concentration and time period (Fig 1). Markers of bone formation (OC, BS-ALP) decreased from time 0 to 4 weeks, peaked at 8-weeks, and then decreased. The marker of bone resorption (DPYR) peaked at 4 weeks and then decreased. There was no association between serum bone marker concentration and treatment group (BMP vs LUC). There were weak but not significant associations between serum bone marker concentration and the 16-week callus grade. Rabbits with a large bridging callus had a higher overall BS-ALP compared to rabbits with smaller non-bridging callus (p=0.05). There was no association between OC or DPYR and the 16-week callus grade or bridging-callas formation. There were significant differences in serum marker concentrations between infected and non-infected rabbits (Fig 2). There was a trend for infected rabbits to have a lower OC at 4 weeks and higher OC at 16 weeks compared to non-infected rabbits (p<0.1). Infected rabbits had a lower BS-ALP at 4 weeks compared to non-infected rabbits (p<0.01). DPYR was higher in infected rabbits at 4, 8, and 16 weeks (p<0.01). There was no association between lysis grade and OC, but there was a decrease in BS-ALP with increase in lysis grade at 4 weeks (p<0.01). Rabbits with a higher lysis grade had a higher DPYR at 4, 8, 12, and 16 weeks (p<0.01). When the combination of serum bone markers was used at 4 weeks, infection could be predicted with an accuracy of 96%.

Discussion: The change in serum bone marker concentration with time was consistent with an initial period of bone resorption followed by bone formation. There was minimal difference in serum bone marker concentrations between rabbits that healed and rabbits that formed a non-union. Variables that may have contributed to this finding include: treatment with Ad-BMP, the use of a sclerosing agent to ensure non-union and development of osteomyelitis, and that rabbits healed by external callus formation rather than defect ossification. However, the results of this study suggest that serum bone markers may be useful for early diagnosis of osteomyelitis associated with fracture healing. The use of multiple markers, early in the course of fracture healing, may be more useful than a single marker at an arbitrary time point. Future studies are required to further evaluate serum bone markers as a method for assessing healing.

Fig 1. A plot illustrating the association between serum bone marker concentration and time averaged over all other variables. The log value of the bone marker concentration is shown on the y-axis and the time (pre-operatively (0), 4, 8, 12, and 16 weeks postoperatively) for each marker is shown on the x-axis. Different letters represent statistically significant differences. The level of significance was p<0.05. OC=osteocalcin, BS-ALP=bone-specific alkaline phosphatase, DPYR=deoxypyridinoline crosslinks.

Fig 2. Plots illustrating the association between serum bone marker concentration and infection. The log value of the bone marker concentration is shown on the y-axis and the time (pre-operatively (0), 4, 8, 12, and 16 weeks postoperatively) is shown on the x-axis. The asterisks represent statistically significant differences between infected and non-infected rabbits. The level of significance was p<0.05. BS-ALP=bone-specific alkaline phosphatase, DPYR=deoxypyridinoline crosslinks, INF=infected, NON=non-infected.