Long-Term Assessment of Bone Regeneration in Nonunion Fractures Treated with Compression-Resistant Matrix and Recombinant Human Bone Morphogenetic Protein-2 in Dogs

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Abstract

Objective The aim of this study was to assess bone density, bone architecture and clinical function of canine nonunion distal appendicular long bone fractures with a defect treated with fixation, compression-resistant matrix and recombinant human bone morphogenetic protein-2 (rhBMP-2).

Study Design Prospective cohort study with dogs at least 1-year post treatment. Computed tomography was performed and quantitative measurements from previous fracture sites were compared with measurements from contralateral limbs. Subjective evaluation included gait assessment and palpation.

Results Six patients met the inclusion criteria. The rhBMP-2 treated bone exhibited higher density at the periphery and lower density in the centre, similar to the contralateral limb. All patients were weight bearing on the treated limb and all fractures were healed.

Conclusion The rhBMP-2-treated bone underwent restoration of normal architecture and density. Acceptable limb function was present in all patients. The results of this study can serve as a basis for long-term response in treating nonunion fractures in veterinary patients.

Keywords

- Bone morphogenetic protein 2
- rhBMP-2
- nonunion fracture
- compression-resistant matrix
- bone regeneration

Introduction

Nonunion after long bone fracture repair can represent a devastating complication for veterinary patients if inadequately treated. Rigid fracture fixation and autogenous bone graft are currently the standard of care to treat nonunion fracture complications.[1] If these treatments fail, it can result in permanent disability and potential loss of the limb. Although the rate of all nonunion fracture complications in veterinary medicine has not been reported in large case series, the rate of complications in humans has been reported as up to 10%.[2] The risk of nonunion is high for fractures of the distal radius and ulna in toy breed dogs due to reduced vascularity compared with large breed dogs.[3] Of nonunion

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fractures reported in dogs, 60% occur in the radius and ulna and 25% occur in the tibia.[4]

Bone morphogenetic proteins (BMP) are multifunctional growth factors that play important roles in embryogenesis and maintenance of adult tissue homeostasis.[5] In the skeletal system, BMP promote new bone formation by stimulating differentiation of pluripotent mesenchymal stem cells into osteoprogenitor cells, osteoblasts and osteocytes.[6] In human orthopaedics, commercially available BMP-2 is U.S. Food and Drug Administration-approved for use in interbody spine fusion, open tibial fractures and oral maxillofacial bone grafting procedures.[7] However, the adverse responses to excessive dosage of BMP in human cervical spinal patients have led to significant controversy surrounding general use of the product,[8] and have hindered further research on its potential benefits and long-term effects. In addition, concerns that BMP treatment does not return bone to normal form and function after remodelling have been reported.[9]

In veterinary medicine, the use of BMP has been successful in the treatment of nonunion long bone fractures,[10–15] mandibular reconstruction[16–19] and arthrodesis.[20–22] Massie and colleagues reported the use of compression-resistant matrix (CRM) infused with recombinant human bone morphogenetic protein-2 (rhBMP-2) in dogs and the short-term study demonstrated that rhBMP-2 is safe and effective, with 9 of 11 treated fractures returning to full function and the remaining 2 fractures having acceptable function.[23]

A carrier is required for optimal delivery and maintenance of BMP concentration at the treatment site. Four major categories of carrier materials include inorganic materials, synthetic polymers, natural polymers and composites.[24] Compression-resistant matrix is a natural polymer composed of collagen sponge with embedded granules of hydroxyapatite and tricalcium phosphate.[25] Several studies report successful clinical outcomes with the use of rhBMP-2 delivered in CRM for osseous defects in mandibular reconstruction and mandibular nonunion fractures.[16–19]

Objective long-term outcome assessment measures including bone mineral density and bone architecture after BMP use in veterinary patients have not been evaluated. Evaluation of bone density and quality has been described using conventional computed tomography (CT) and Hounsfield units (HU) measurements.[26,27] The objective of this study was to use CT to evaluate bone density and architecture of distal appendicular long bone that was at least 1-year post-treatment for a nonunion fracture with a defect using fixation, CRM and rhBMP-2. A second objective of this study was to evaluate long-term subjective clinical outcome.

Materials and Methods

Dogs that had undergone treatment of a nonunion distal appendicular long bone fracture with osseous defect using internal fixation, CRM and rhBMP-2 at UC Davis Veterinary Medical Teaching Hospital were identified using a medical record search. Owners were contacted via telephone to enroll eligible dogs in a prospective cohort study using CT to evaluate bone density and architecture. All dogs had pre-, post- and follow-up radiographs taken as part of the surgical management (►Fig. 1) and all dogs were at least 1 year post-treatment.[28] A defect was defined as an osseous fracture gap based on intraoperative observation of a fracture gap filled with fibrous soft tissue as recorded in the surgical report and

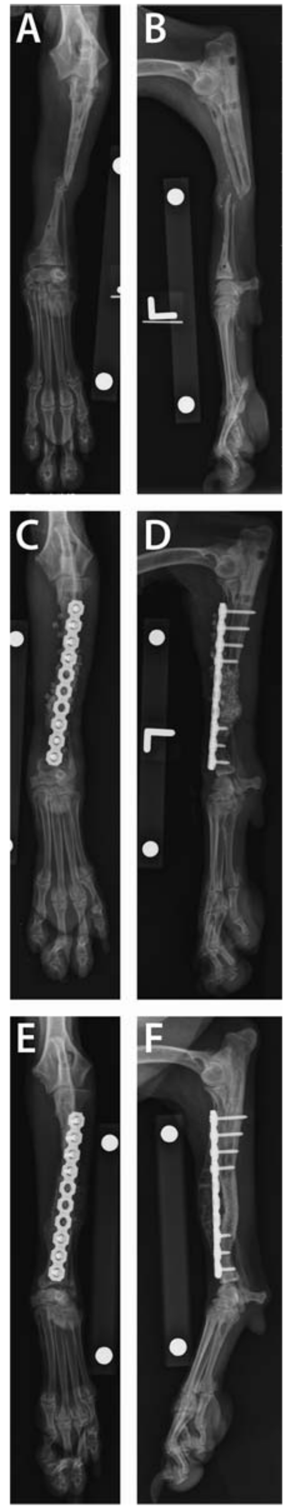

Fig. 1 Cranial–caudal and lateral–medial radiographs taken prior to surgery (**A, B**), immediately postoperatively (**C, D**) and at 8 weeks following surgery (**E, F**).

postoperative radiographic appearance of a gap. Exclusion criteria included noncanine species, cases that were less than 1-year post-treatment, cases that had no defect or history of nonunion, cases with humeral or femoral fractures and nonfracture cases that underwent arthrodesis. The study protocol was reviewed and approved by the UC Davis Institutional Animal Care and Use Committee and written owner consent was obtained.

At study enrolment, participants were presented for repeat evaluation and CT examination. Recorded clinical data included signalment, affected bone, defect size, time from rhBMP-2 to CT scan, number of previous surgeries, previous fixation methods used and type of fixation used in conjunction with rhBMP-2. Bone defect of the nonunion fracture was measured on postoperative radiographs following calibration using a DICOM viewer (Horos, Geneva, CH) and the ratio of defect was calculated using the contralateral bone as the reference.

All patients underwent a complete physical and orthopaedic examination. Still images and gait videos were obtained. Subjective assessment of clinical outcome in the rhBMP-2 treated limbs was performed. Full function was defined as restoration to full intended level and duration of activities and performance from preinjury status without medical intervention. Acceptable function was defined as restoration to intended activities and performance from preinjury that was limited in level or duration, or required medical intervention, or a combination of both.[28] The outcome definition was expanded for the current study such that return to normal activity with no restrictions, full weight bearing at a stand and walk and lack of pain on palpation and range of motion of the surgical limb were classified as full function, while dogs with acceptable function could have intermittent change in limb use or altered range of motion.

The dogs were sedated using 5 μg/kg dexmedetomidine (Zoetis, Parsippany, New Jersey, United States) and 0.2 mg/kg butorphanol (Zoetis, Parsippany, New Jersey, United States) intravenously, and a CT scan of the treated and contralateral limbs was performed using a 16-slice CT scanner (LightSpeed CT scanner, GE Healthcare, Waukesha, Wisconsin, United States). Image acquisition parameters included pitch of 0.562, in 0.6 mm slices, with a field of view of ~12 cm. Image reconstruction was performed using a bone algorithm in a 512 × 512 matrix. The CT images were reviewed using a DICOM viewer with ability to perform three-dimensional multiplanar reformats and maximal intensity projections (Horos, Geneva, CH). All CT scans were subjectively reviewed by a board-certified radiologist (MS) and evidence of bone regeneration and healing status was recorded. The overall bone length of the surgical and contralateral bone was measured and recorded. For quantitative measurements, a transverse CT image was centred over the previous fracture site of the rhBMP-2 treated bone (►**Fig. 2**). The previous fracture site was identified by measuring the location on the postoperative radiographs and identifying the same location along the bone on the CT image. A transverse CT image was selected at the equivalent location on the contralateral (control) limb. On each transverse image, the area of the bone was calculated using a freeform region of interest (ROI) tool that outlined the perimeter of the bone. Bone density was measured by drawing a medio-lateral and a cranio-caudal line that bisected the bone to generate a linear density profile which was expressed as HU. If a bone plate was present, the medio-lateral and cranio-caudal lines excluded bone directly under the plate to minimize artefact. The maximum density values at medial, lateral, cranial and caudal margins were collected and defined as cortical bone density. The minimum density value of each line through the centre of the bone was collected and defined as medullary bone density. The ROI and density measurements were repeated three times by the same observer (AC). Averages of these measurements were used for analysis. Area of the bone, cortical bone density and medullary bone density were compared between rhBMP-2 treated bone and contralateral bone using a mixed model analysis of variance (ANOVA) (SAS 9.3, SAS Institute Inc, Cary, North Carolina, United States) with post-hoc pairwise comparisons. The effect of the bone having a bone plate or not during the CT scan was included in the ANOVA model. The individual dog was treated as a random effect and the location of the measurement along the bone was treated as a repeated effect. Normality of the ANOVA residuals was assessed using the Shapiro–Wilk statistic. When residuals were not normally distributed, a repeated measures ANOVA was performed using the ranks for affected variables. Statistical significance was set at $p < 0.05$. Spearman correlation was used to determine if area and density outcomes were related to time duration from rhBMP-2 treatment to CT scan or related to dog body weight. If there was a significant relationship at $p < 0.05$, then the correlation coefficient (R) was reported.

Results

Study Population

Of 35 patients treated using rhBMP-2 (Medtronic, Minneapolis, Minnesota, United States) at the time of the study, 10 dogs satisfied the inclusion criteria. Twenty-five cases were excluded due to noncanine species ($n = 3$), lack of defect or nonunion fractures ($n = 8$), humeral or femoral fractures ($n = 12$) and arthrodesis surgery ($n = 2$). Of the 10 eligible dogs, six dogs were prospectively enrolled in the study. Four cases were not enrolled due to significant respiratory and cardiac disease ($n = 1$), loss to follow-up ($n = 2$) and owner's inability to travel ($n = 1$). Out of the six dogs enrolled, four dogs had undergone fracture stabilization of the radius, while two dogs had undergone stabilization of the tibia. Two of the dogs enrolled in the study had undergone fracture surgery on the contralateral radial bone using bone plate and screws and recovered from this surgery without complications. One of these dogs underwent implant removal on the contralateral limb greater than 1 year prior to CT scan, while the contralateral limb with the bone plate in place was not used for statistical analysis. The short-term outcome of one dog had previously been reported.[23]

The median [range] age at the time of rhBMP-2 treatment and CT scan was 2 [1–11] years and 4.5 [2–12] years

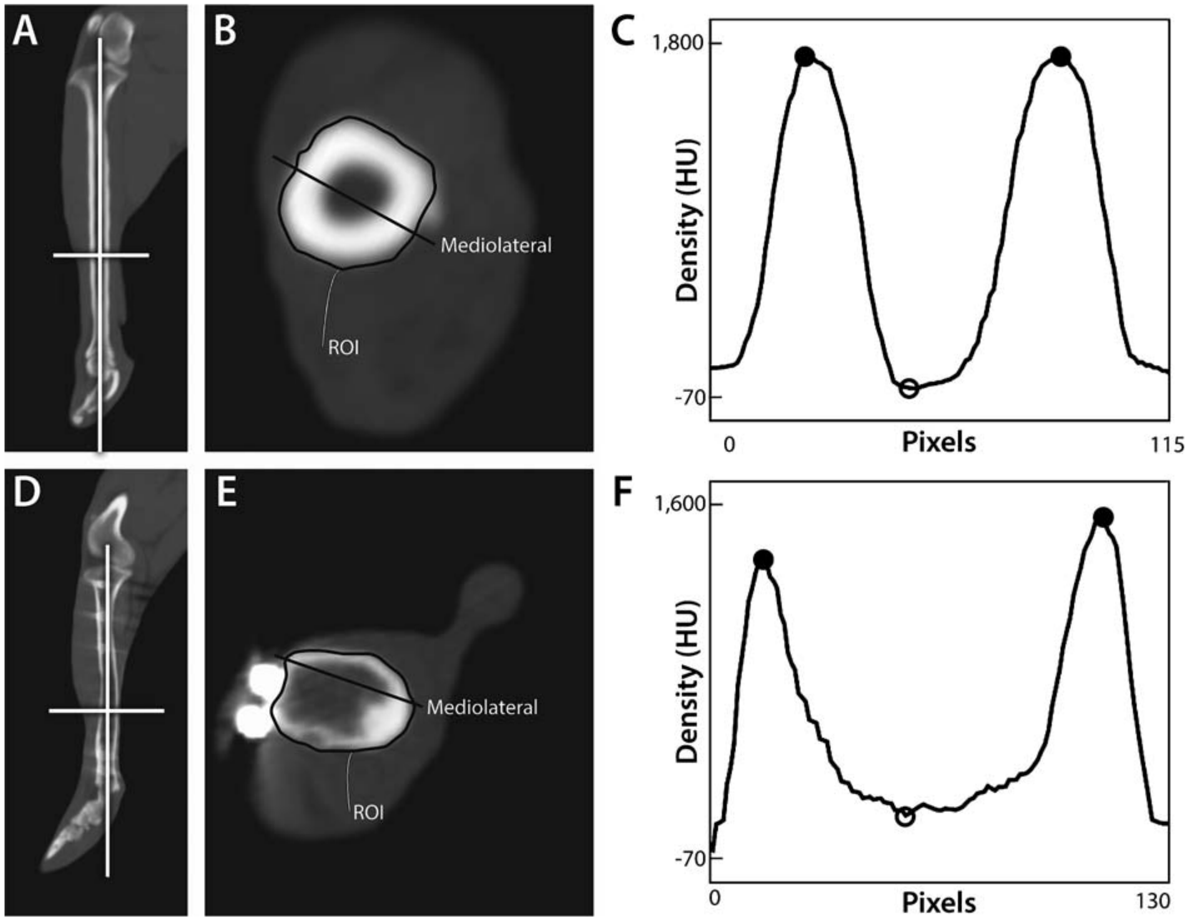

Fig. 2 Images of contralateral (control) bone (**A–C**) and recombinant human bone morphogenetic protein-2 treated bone (**D–F**). A and D depict the level along the bone at which the transverse image was generated. This is at the fracture site in image D and the corresponding level in the contralateral bone (**A**). B and E depict the orientation of the mediolateral density line and the region of interest (ROI) outlined. The ROI was used to calculate the area of the bone. Using the mediolateral line, graphs (C and F) were generated depicting the density of cortical bone (solid circle) and the density of medullary bone (open circle). HU, Hounsfield unit.

respectively. The median [range] defect size was 2.1 [0.6–2.8] cm with a median [range] ratio of bone defect to length of control bone of 0.26 [0.08–0.39]. All rhBMP-2 treated limbs were shorter than the contralateral limbs, with a median [range] difference in limb length of 1.15 [0.2–2.9] cm (►**Table 1**). The median [range] number of surgeries prior to fixation and use of rhBMP-2 was 4.5 [1–7] surgeries and previous fixations used included bone plates, external skeletal fixators and an interlocking nail (►**Table 2**). The median [range] time from rhBMP-2 treatment to CT scan was 571 [379–1467] days. All patients had bone plates placed in bridging fashion at the time of surgery. Three of the four dogs with radial fractures had a plate spanning the antebrachiocarpal joint. Three dogs (2 radial fractures, 1 tibial fracture)

Table 1 Summary table of dog signalment, bone involved and the defect sizes

Patient	Breed	Weight (kg) at time of rhBMP-2 application	Sex	Bone	Control bone length (cm)	Affected bone length (cm)	Defect size (cm)	Bone to defect ratio
1	Jack Russell	5.2	MC	Tibia/fibula	11.4	9.5	3.5	0.31
2	Yorkshire Terrier	2.0	FI	Radius/ulna	6.5	5.3	1.8	0.28
3	Xoloitzcuintli	10.6	MC	Radius/ulna	12.2	9.3	2.8	0.23
4	Yorkshire Terrier	1.7	MC	Radius/ulna	7.4	6.9	0.6	0.08
5	Yorkshire Terrier	1.1	FI	Radius/ulna	6.2	5.1	2.4	0.39
6	Mixed breed	4.3	MC	Tibia/fibula	12.2	12	1.5	0.12

Abbreviations: FI, female intact; MC, male castrated; rhBMP-2, recombinant human bone morphogenetic protein-2.

Table 2 Summary table of number of surgeries prior to rhBMP-2 application, type of fixation, implant position and presence of bone plates at time of CT

Patient	Previous surgeries	Fixation with rhBMP-2	Location of plate	Plate on study limb at time of CT	Plate on contralateral limb at time of CT
1	1	2.0 mm 13-hole LCP	Medial	Yes	No
2	2	1.5 mm 12-hole locking adaptation plate	Cranial	No	No
3	7	2.4 mm 10-hole DPS mandibular reconstruction plate	Cranial	Yes	No
4	5	1.5 mm 12-hole locking adaptation plate	Cranial	Yes	No
5	4	1.5 mm 12-hole locking adaptation plate	Cranial	No	Yes
6	5	2.0 mm 20-hole craniomaxillary locking plate 2.4 mm 14-hole LCP	Cranial and medial	No	No

Abbreviations: DPS,—; CT, computed tomography; LCP, locking compression plate; rhBMP-2, recombinant human bone morphogenetic protein-2.

had undergone implant removal prior to enrolment in the study and subsequent CT scan.

Computed Tomography

Subjective Findings

All treated fractures were healed at the time of CT scan with presence of new bone formation leading to complete union. Minimal artefact was noted at the fracture sites from the bone plates present and no screws were present at the fracture site (►**Fig. 2**). The new bone area appeared to have higher density at the periphery in five of the six dogs. The central part of the bone was more lucent compared with the outer part but contained a wispy mineral opacity. In the remaining dog, the bone was small with a triangular shape and distinction between the central and peripheral parts was not possible. In one of the four dogs with a radial fracture, the new bone formation led to complete encasement of the bone plate within the bone. In the remaining three dogs with previous radial fracture, there was partial to complete carpal ankylosis defined as bone crossing the joint.

Objective Measurements

The median [range] area of bones in rhBMP-2 treated bone (42 mm^2 [7–102]) compared with contralateral bone (45 mm^2 [13–53]) was not statistically different ($p = 0.400$) (►**Fig. 3**A). The median density of the entire cross section was not significantly different in rhBMP-2 treated bone (1198 HU [782–1407]) compared with contralateral bone (1326 HU [1,161–1,518], $p = 0.934$) (►**Fig. 3**B). The median cortical density was not significantly different for rhBMP-2 treated bone (1,483 HU [1,182–1,746]) compared with contralateral bone (1,813 HU [1,625–2040], $p = 0.431$) (►**Fig. 3**C). Similarly, the median medullary density was not different for rhBMP-2 treated bone (414 HU [73–1,368]) compared with contralateral bone (−45 HU [−141–979], $p = 0.083$) (►**Fig. 3**D).

The area, median maximum cortical density and median minimum medullary density of individual dogs are presented in ►**Fig. 4**. Based on subjective interpretation, dogs greater than 2.5 kg (dogs 1, 3, 6) demonstrated larger area, decreased cortical density and increased medullary density in rhBMP-2 treated bone when compared with contralateral bone. Dogs less than 2.5 kg (dogs 2, 4) showed similar area, cortical and medullary density between rhBMP-2 treated bone and contralateral bone (►**Fig. 4**). When analysing all bones, significant correlation was found between area and body weight ($R = 0.73$, $p = 0.015$) and between medullary density and body weight ($R = -0.69$, $p = 0.029$) (►**Fig. 5**). The magnitude of increase of area with body-weight (slope of the regression line) was significantly larger ($p = 0.0006$) for rhBMP-2 treated bones ($R = 0.83$, $p = 0.041$) compared with contralateral bones ($R = 0.6$, $p = 0.285$). Time duration did not affect area and density.

Clinical Outcome

At the time of re-examination, all dogs had unrestricted activity. Three dogs returned to full function and three to acceptable function. All dogs were weight bearing on the affected limb at a walk and trot during orthopaedic examination. Three of six dogs would intermittently lift the treated limb up when standing still. All dogs were subjectively assessed as being pain free on palpation of long bones and joint range of motion.

Discussion

The formation of outer cortical bone and inner medullary bone as seen on CT suggests that rhBMP-2 treated bone undergoes regeneration, ossification and remodelling at the fracture site when a defect is present. Although no statistical differences were detected, rhBMP-2 treated cortical and medullary bone density followed different trends compared with the contralateral bone. These differences in density may have clinical significance as it relates to mechanical properties observed in cortical and medullary bone. Cortical bone exhibits higher density, stiffness and strength compared with cancellous bone; however, it is more brittle and may fail with very little plastic deformation.[29] In contrast, cancellous bone is more compliant and can withstand higher strains prior to deformation. The implication of these characteristics between cortical and cancellous bone in relation

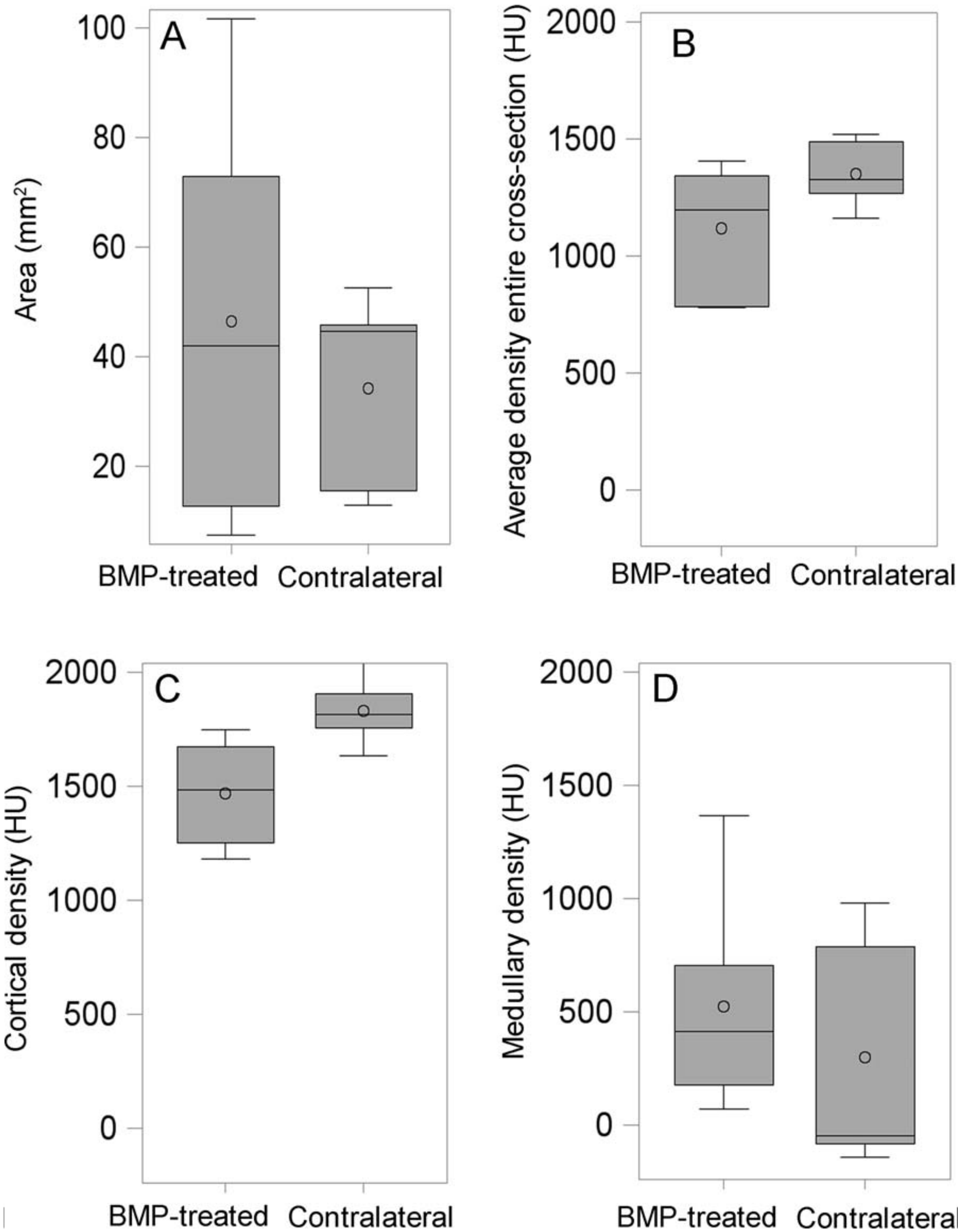

Fig. 3 Boxplots of recombinant human bone morphogenetic protein-2 (rhBMP-2) treated bone at the fracture level versus contralateral bone at the corresponding level comparing (**A**) area of bone, (**B**) density, (**C**) cortical density and (**D**) medullary density. No statistical difference was detected. HU, Hounsfield unit.

to the properties of rhBMP-2 treated bone is not well established. Differences in bone density and composition could affect quality and function of rhBMP-2 treated bone, which warrant further research to assess differences in biomechanical properties between rhBMP-2 and non-rhBMP-2 treated bone.

Restoration to full function was not achieved in all dogs as intermittent lifting of the treated limbs was observed in three patients when standing and classified as acceptable function but all patients had restoration of normal activity level and no evidence of pain on subjective evaluation. Differences in limb length observed between rhBMP-2

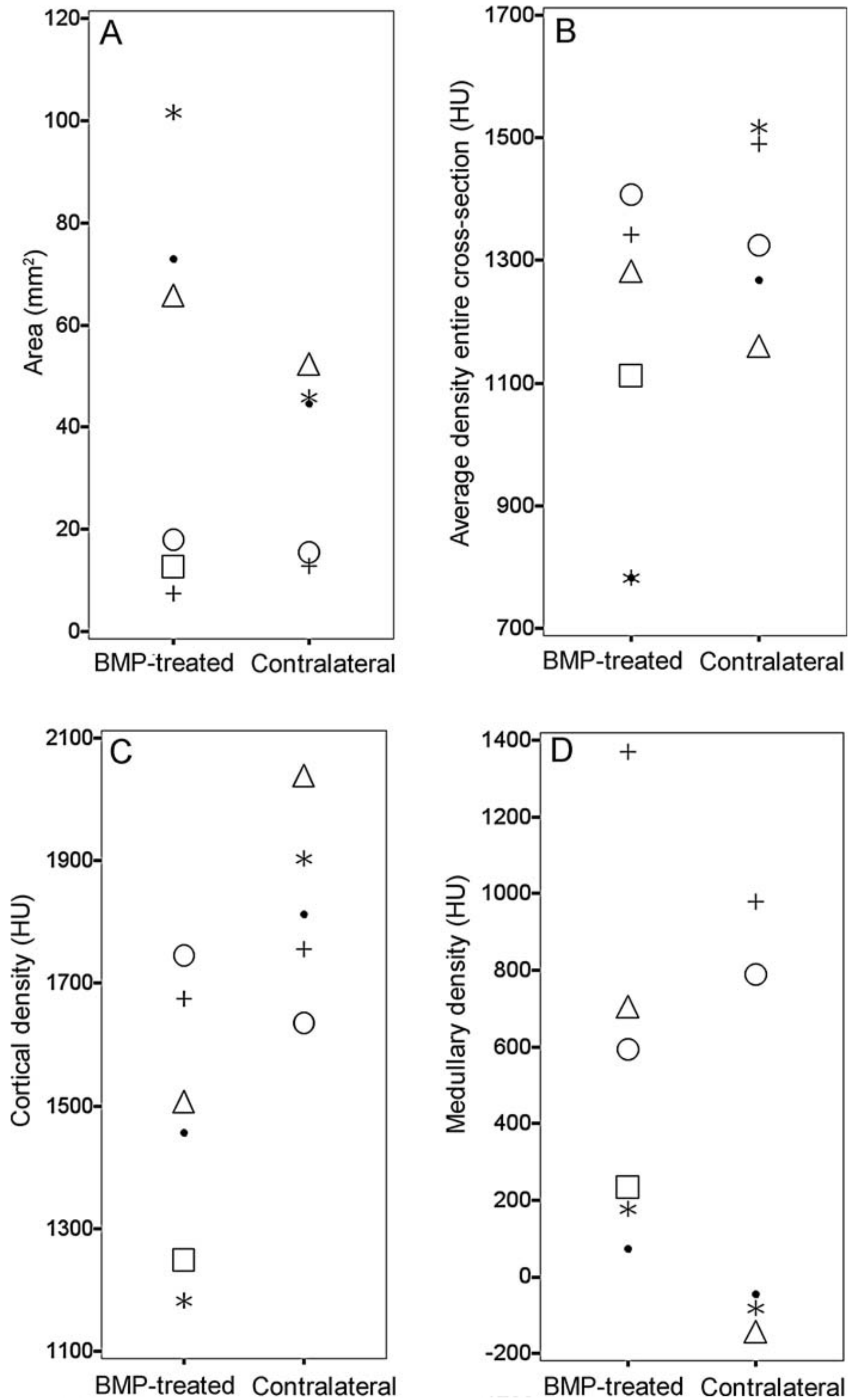

Fig. 4 Boxplots of individual dogs comparing recombinant human bone morphogenetic protein-2 (rhBMP-2) treated bone at the fracture level versus contralateral bone at the corresponding level—(**A**) area of bone, (**B**) average density, (**C**) cortical density and (**D**) medullary density. Individual dogs are denoted by symbols as dog 1 (dot), dog 2 (circle), dog 3 (asterisk), dog 4 (plus sign), dog 5 (square) and dog 6 (triangle). HU, Hounsfield unit.

treated limbs and contralateral limbs may have contributed to a mechanical lameness and intermittent lifting of the treated limb at stance. Ostectomies of 10, 15 and 20% in the mid-diaphyseal femur in dogs have been shown to result in significant increase in standing angles of the stifle on the operated limb and decrease in standing angles of the stifle on the contralateral limb.[30] There has been no published assessment of effects of thoracic limb shortening in dogs. In addition to differences in limb length, three of the dogs had ankylosis of the carpal joint, which can contribute to a

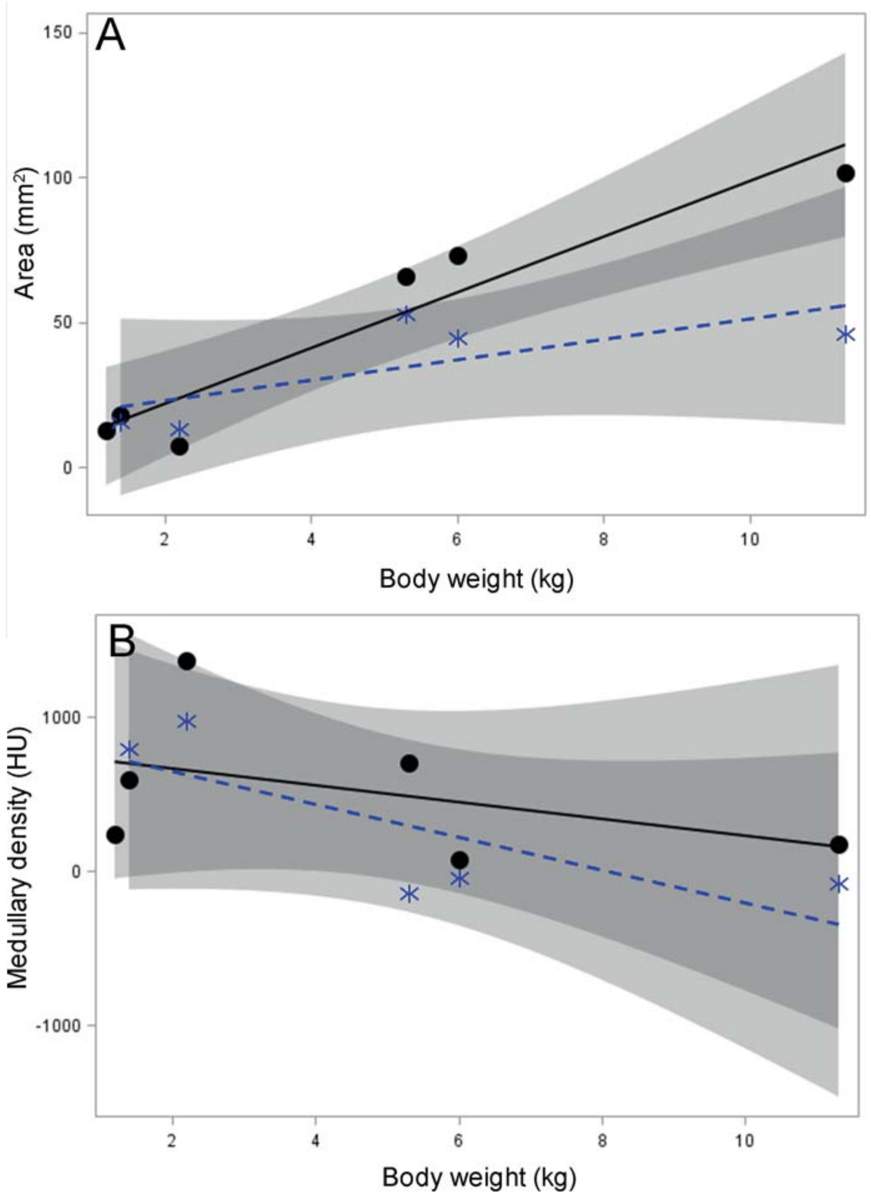

Fig. 5 Regression models of the area of bone at the fracture level compared with body weight (**A**) and medullary density compared with body weight (**B**) in recombinant human bone morphogenetic protein-2 treated dogs (solid line, solid circles) and contralateral bone (dashed line, asterisks). HU, Hounsfield unit.

mechanical lameness. Restricted carpal range of motion has been associated with reduced vertical impulses, decreased braking action and increased shoulder joint angulation.[31,32]

In rhBMP-2 treated bone, an increase in body weight was significantly correlated with larger area of bone whereas contralateral bone was not. Smaller dogs may have less increase in area of rhBMP-2 treated bone due to decreased intraosseous and soft tissue vascular density of the antebrachium in small- and toy-breed dogs.[3] This may be further exacerbated by multiple surgeries. In spite of this, small breed dogs showed evidence of bone formation and restoration of bone architecture in regions of previous nonunion fracture with osseous defect. All patients underwent application of small sized bone plates in bridging fashion using long working lengths. Lower area moment of inertia and increased functional working length of implants result in increased compliance and potential increased risk of plate bending or plastic deformation. Given that there were no complications of plate breakage, this likely demonstrates early soft callus formation, rapid bone formation and increased stability of the repair associated with use of rhBMP-2.

In this study, cases were enrolled if an osseous defect of any size was present. The smallest defect recorded was 6 mm and this represented 8% of the overall length of the bone based on the contralateral radial length. Prior to surgery, all of the dogs enrolled were considered to have a defect nonunion based on radiographic examination. In addition, all cases required removal of dead bone intraoperatively, further contributing to the defect. Currently, there are no defined criterion for critical osseous defect in dogs and the decision to use rhBMP-2 in these defect, non-union cases presented in this study was based on overall bone quality on preoperative radiographs, the number of previously failed surgeries, and intraoperative bone quality.

One of the patients developed excessive bone at the site of rhBMP-2 application, leading to encasement of the plate within the bone. This could result in complications if the plate needs to be removed. Although excessive or ectopic bone formation has not been reported in previous veterinary studies using BMP,[16,23,33] it is a well-documented side effect of BMP used in spinal fusion procedures.[8] This could be related to dose, carrier system, or leakage of BMP outside of the implant site.

The use of CT to evaluate fracture healing is particularly useful in challenging cases with equivocal radiographic findings. In a study evaluating bone healing in human orthopaedic patients, radiographs underestimated or overestimated the extent of bone healing in 37% of patients.[34] The use of CT provides a more objective assessment of healing bone by allowing quantitative measurements of properties such as bone mineral density and callus volume. Animal studies assessing bone strength have demonstrated strong correlation with bone mineral density as measured on CT.[35,36] Metal implants can generate artefacts that could have compromised the measurements.[37] In the current study, there was minimal artefact noted, likely due to the small size of the bone plates used. A difference in density between the periphery and the centre (►**Fig. 2**) of the bone was easily visible and measured. The line density profile (►**Fig. 2**) shows mild irregularity in the line, which is secondary to mild streaking artefact from the plate, but there is minimal effect on the overall shape of the line density profile, indicating that measurements are not affected. Therefore, the presence of bone plates in three patients over the fracture site would have minimal effect on the overall assessment of bone regeneration and quality.

Limitations of the study include small sample size with a heterogenous population of dogs. Two of six dogs had undergone previous standard fracture repair with internal fixation of the contralateral bone, which may have affected the bone architecture and density of the control bone. However, this limitation represents a real-life clinical situation and, as one of the objectives of this study was to report on the clinical outcome, the dogs were enrolled. The study population was limited by the criteria which aimed to evaluate dogs with nonunion distal appendicular long bone fractures with an osseous defect and as such, many cases were excluded. Despite the limitations, the results show that bone density and architecture of canine nonunion fracture bone treated with rhBMP-2 regeneration and clinical function, despite multiple surgeries and limb shortening, were acceptable to full in all patients. Therefore, the use of rhBMP-2 for nonunion fractures should be considered as a viable technique with long-term acceptable outcomes.

Authors' Contributions

All authors contributed to the development of study design. ASK performed the surgery on all of the patients. AC and ASK evaluated the clinical cases. MS, AC, BF, ASK and T.G contributed to the acquisition of data, data analysis and interpretation. AC and BF developed the main manuscript and all the authors contributed to the review and approval of the manuscript prior to submission.

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Conflict of Interest

None declared.

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