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CASE REPORT
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Augmentation of Distal Antebrachial Non-union Healing in a Bitch with Local Application of Erythropoietin

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ABSTRACT

***Background*:** The clinical management of non-unions relies on osteogenic, osteoinductive, osteoconductive properties of autologous bone grafts. Recently, the augmentation of fracture healing assumed paramount importance in modern orthopaedics, particularly in the management of non-unions with extensive bone defects. This report presents a clinical case of a Pomeranian with atrophic non-union of distal antebrachial bones treated with cancellous bone graft mixed with erythropoietin. The autologous grafts provides live osteogenic cells, whereas erythropoietin apart from attracting osteoblasts at the fracture site, improved blood supply due to its angiogenic potential.

***Case*:** A 11-month-old intact bitch Pomeranian, was referred for orthopaedic examination 1 day after traumatic injury following jump from a height. The patient had fracture of distal antebrachial bones accompanied with grade IV weight-bearing lameness and swelling, as well as severe pain and crepitus on palpation. The bitch was initially treated with dynamic compression plate and screws. Thirty-six days after the osteosynthesis, the patient was brought with severe pain and swelling of the operated limb, high-grade lameness, osteolytic areas due to implant loosening, severe thinning of compact bone and bone callus paucity. During the operative revision, existing implants were removed. The bone holes from screws and the bone defect were filled with autologous cancellous bone harvested from the proximal humerus, mixed with recombinant human erythropoietin for stimulation of bone healing. After the surgery, the limb was immobilized with a rigid splint and the patient spent 10 days in a cage for restriction of movements. The post operative period was smooth and as early as the 12th post-operative day, the animal was discharged from the hospital with very good weight bearing with the limb, absence of lameness, pain and swelling. Radiographs demonstrated complete healing of the 2 bones and filling of bone holes from cortical screws.

***Discussion*:** Non-union is one of the most serious complications of fractures in dogs. The reported great percentage of antebrachial non-unions (up to 60% of cases) requires application of a method for bone healing promotion. Bone grafting is a preferred approach, in particular the use of autologous bone grafts that are well integrated in the host bone with minimum risk of infection or displacement. The autologous cancellous bone graft in the dog was applied together with erythropoietin in order to promote bone healing on the basis of previous research data confirming its osteoinductive and angiogenic potential. It stimulates the differentiation of mesenchymal stem cells into osteoblasts, thus improving bone regeneration and speeding bone healing, modulates inflammation by antagonising pro-inflammatory cytokines. Experimental studies in mice, rats and rabbits having explored the local bone regenerating effect of erythropoietin, either alone or with a bone substitute provided histological evidence that erythropoietin stimulated angiogenesis and potentiated the effect of bone substitute and mineralisation at the bone defect site, and increased fibrous tissue and blood vessels formation in treated in comparison to untreated bone defects. In the presented clinical case of atrophic non-union with possibly avascular bone ends, the osteogenic and angiogenic potential of erythropoietin, mixed with the autologous cancellous bone graft for filling of bone defect and bone holes from cortical screws, resulted in bone healing over a very short period with excellent clinical outcome. Therefore, erythropoietin appears a promising adjunct to the functional therapy for bone non-union regeneration.

Keywords: Pomeranian, dog, bone graft, fracture, traumatic injury, atrophic antebrachial non-union, cancellous autograft.

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INTRODUCTION

Radioulnar fractures are often encountered in dogs from toy breeds [6,12]. Also, half of registered radioulnar post operative complications are registered in dogs weighing < 6 kg [10].

The clinical management of non-unions consists in adjustment of the biological environment in case of atrophic non-unions and adjustment of the mechanical environment for hypertrophic non-unions. Atrophic non-unions are established in the early fracture healing stages and are radiologically manifested by callus paucity at the fracture site [19].

All non-unions require surgical treatment [3,18]. Autologous bone grafting is the gold standard due to its osteogenic, osteoinductive, osteoconductive properties. Recently, fracture healing augmentation assumed paramount importance in orthopaedics, particularly in non-unions with large defects [16]. Two main groups of compounds are helpful in this regard: bone substitutes as β-tri-calcium phosphate [20], and sources of growth factors as recombinant human bone morphogenetic protein-2 [4], platelet-rich plasma [5], autologous adipose derived stem cells [11]. To facilitate vascular in-growth, substances with angiogenic potential have been also explored. The combination of erythropoietin and a bone graft was found to accelerate integration with the host bone [22].

To the best of our knowledge, there are no reports on the clinical application of erythropoietin as promoter of non-union bone healing in dogs. This was the incentive to describe a clinical case in a dog with antebrachial non-union, where the application of autograft together with erythropoietin resulted in very rapid bone healing (over 12 days).

CASE

A 11-month-old intact bitch Pomeranian, body weight 1.2 kg was referred to the University Veterinary Hospital of the Faculty of Veterinary Medicine, Stara Zagora, Bulgaria 1 day after traumatic injury following jump from a height. Physical and blood laboratory examinations were normal. The orthopaedic examination found out grade IV weight-bearing lameness and swelling, as well as severe pain and crepitus on palpation. Following deep sedation with medetomidine hydrochloride[1] [Dorbene vet® 1 mg/mL - 0.075 mg/kg i.m., single dose] and ketamine hydrochloride[2] [Anaket® 100 mg/mL - 7.5 mg/kg i.m., single dose], mediolateral and craniocaudal radiographs of the affected limb were taken with a stationary X-ray machine[3]. Radiographs demonstrated linear radiolucency in the metaphyseal part of the radius and the ulna with mild dislocation of fractured bone fragments (Figure 1).

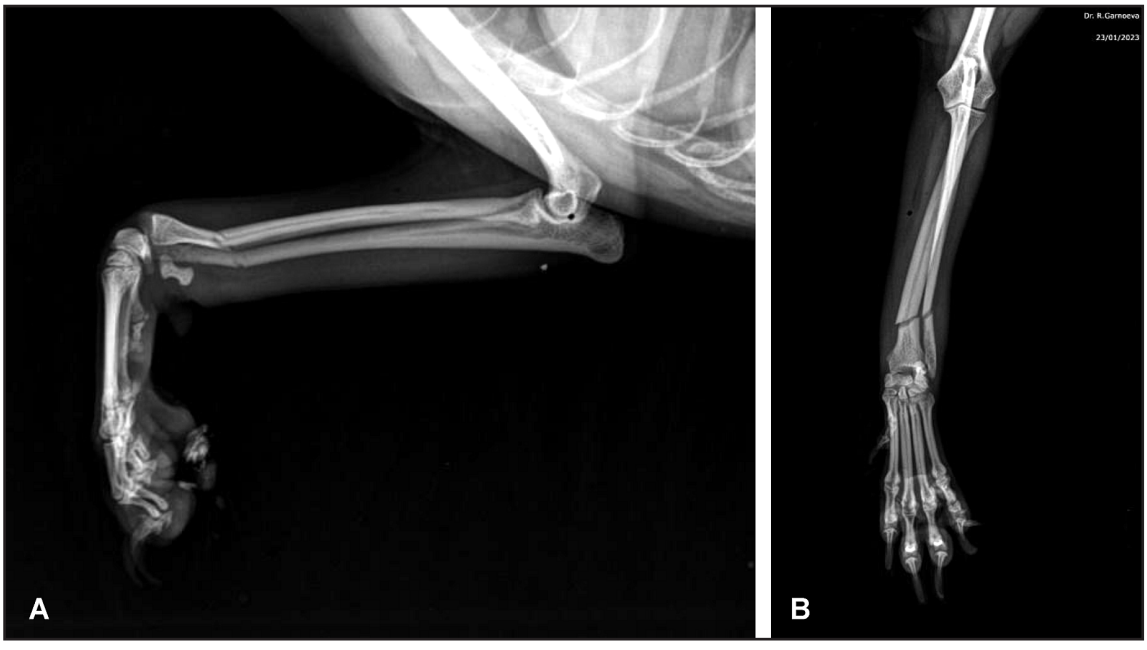

Figure 1. Mediolateral (A) and craniocaudal (B) radiographs of antebrachial bones, showing linear radiolucency in the metaphyseal part of bones.

Osteosynthesis with a dynamic compression plate was made. The anaesthesia protocol included preanesthesia with acepromazine maleate[4] [Neurotranq® 10 mg/mL - 0.2 mg/kg, i.m., single dose] and buprenorphine[5] [Vetergesic® 0.3 mg/mL - 0.01 mg/kg, i.m., single dose] applied in m. quadriceps femoris. Thirty minutes later, ipropofol[6] [Propofol Fresenius® - 5 mg/kg, i.v., single dose] was used for induction. Anaesthesia was maintained with isoflurane[7] [Isoflurin® - 1000 mg/g, 1.5-2 vol%] with 100 % O_2 flow and fluid therapy with 10 mL/kg/h Ringer lactate[8] [Ringer Braun].

After craniomedial approach to the distal antebrachium and incision of the deep antebrachial fascia, m. extensor carpi radialis and m. flexor carpi radialis were separated. After visualization of the fracture line and alignment of bone fragments, osteosynthesis of the radius with a plate and 7 screws (2 in the distal and 5 in the proximal fragment) was carried out (Figure 2).

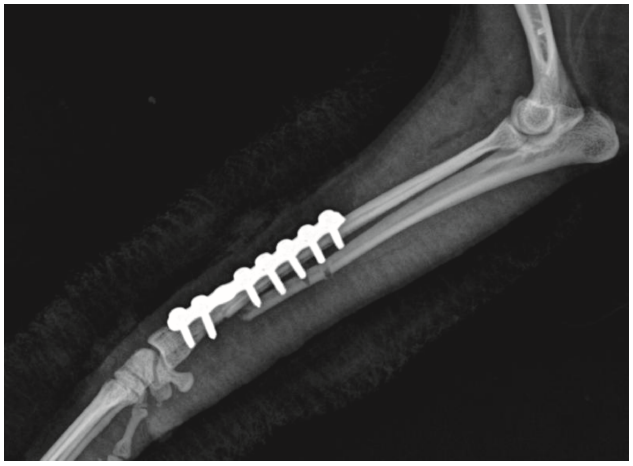

Figure 2. Mediolateral radiograph of antebrachial bones immediately after dynamic compression plate osteosynthesis.

The control orthopaedic examination by the 16th post-operative day revealed abnormal use of the limb, lameness and pain on palpation. Mediolateral control radiograph showed atrophy of bone ends. The distal fragment was thinned and cone-shaped (Figure 3). During the 2nd control examination (36 days following the osteosynthesis), the bitch exhibited high-grade lameness, pain and limb swelling. Radiographic findings consisted in osteolytic areas due to implant loosening, severe thinning of compact bone and scarce amount of bone callus (Figure 4).

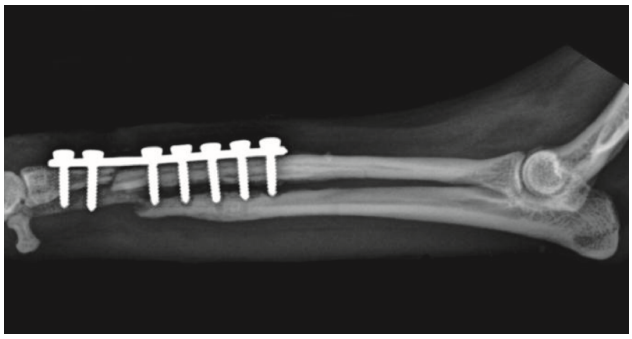

Figure 3. Mediolateral radiographs of antebrachial bones, 16 days after the surgery.

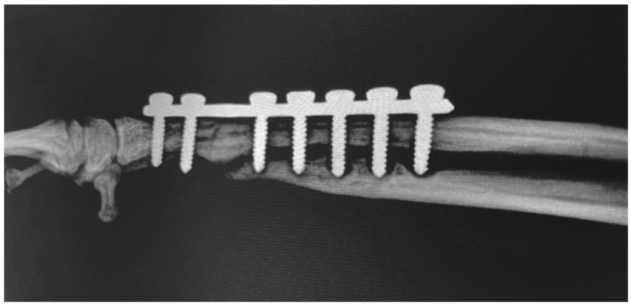

Figure 4. Mediolateral radiographs of antebrachial bones, 36 days after the surgery showing osteolytic areas around the screws due to their loosening, severe thinning of the bone compacta and bone callus paucity.

A 2nd operative intervention was performed using the aforementioned anaesthesia protocol. The plate and the screws were removed following medial approach to the distal radius (Figure 5). In order to stimulate bone healing, the visible bone defect and the holes from the screws were filled with autocancellous bone, mixed with 1,000 IU recombinant human erythropoietin[9] [Binocrit - 1,000 IU/0.5 mL]). The autocancellous bone graft was harvested with a bone curette from the proximal humeral metaphysis after craniolateral approach (Figure 6). Immediately after the revision surgery, the operated limb was immobilized with a rigid splint and bandaged. A total of 10 days of cage rest were prescribed for restriction of movements. To prevent post-operative infection, ceftriaxone[10] [Tercef - 30 mg/kg i.m., SID] was injected for 7 consecutive days. Pain was controlled by injection of meloxicam[11] (Meloxidolor - 0.3 mg/kg, s.c., SID] for 3 days. After 1 week of the surgery, no signs of infection were present and the patient began using the operated limb.

After 12 days of the surgery and before the discharge of the patient from the hospital, the splint was

removed. Control mediolateral radiography showed complete filling of the fracture line in the radius and the ulna with bone callus, as well as filling of bone holes for the screws (Figure 7). Computed tomography with a 32-slice CT scanner[12] (slice thickness 1 mm, exposure data 120 kV, 80 mA, 400 ms) was also performed. DICOM-images were imported into Syngo Via View & Go software[12]. The 3-dimensional reconstruction confirmed the excellent filling of fracture line and screw holes (Figure 8).

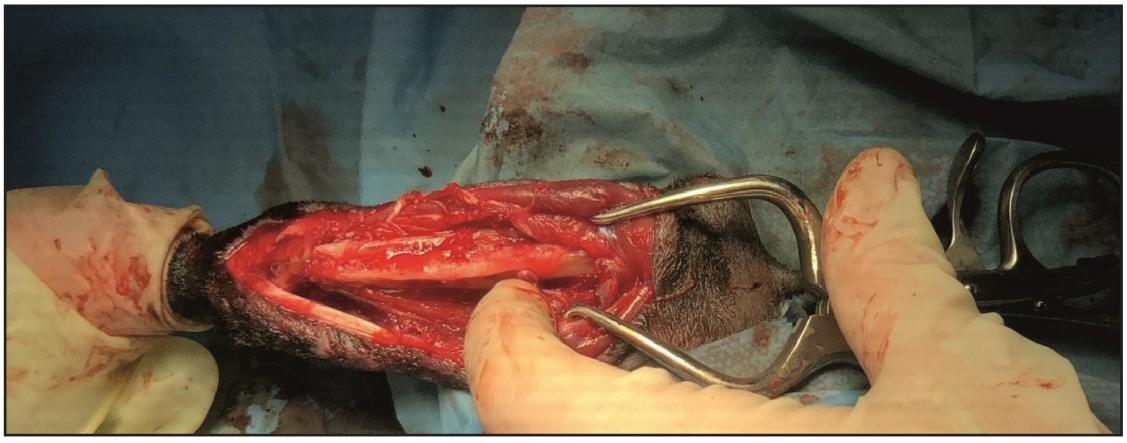

Figure 5. The radius after removal of the plate and the screws.

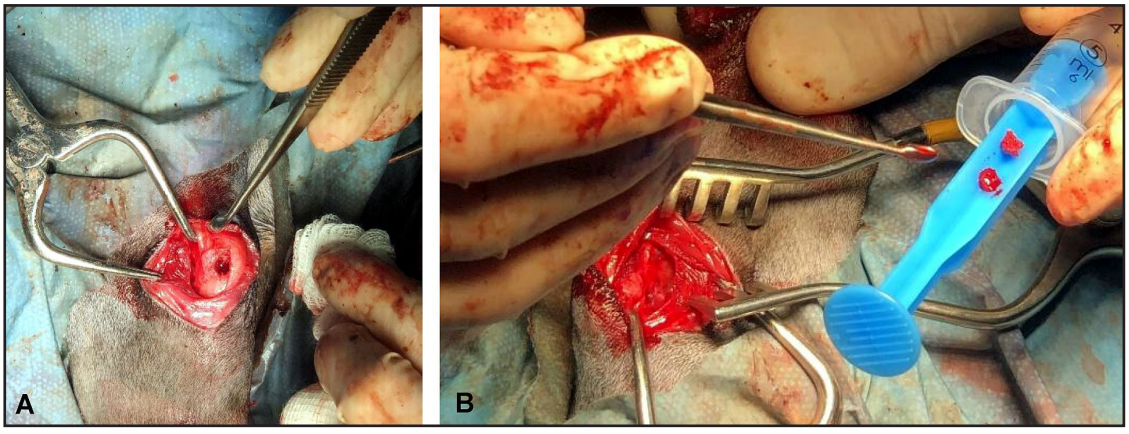

Figure 6. Harvesting of autocancellous bone from the proximal methaphysis of the humerus.

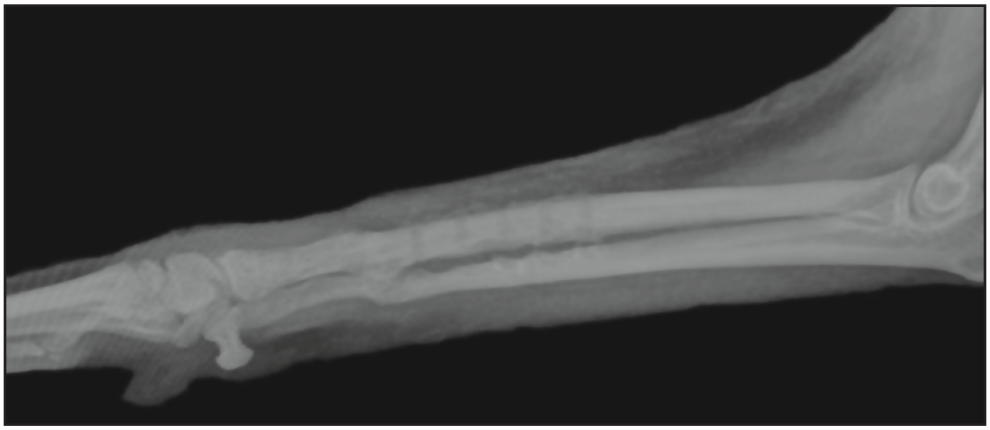

Figure 7. Mediolateral radiographs of antebrachial bones, 12 days after application of autocancellous bone graft mixed with erythropoietin in the bone defect and holes from cortical screws.

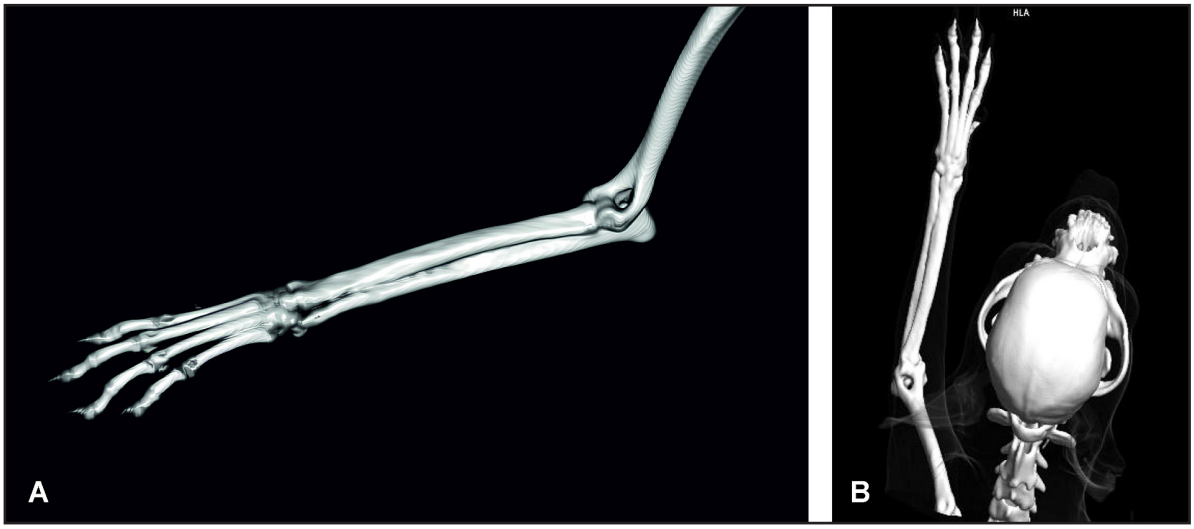

Figure 8. Three-dimensional computed tomography reconstruction, 12 days after application of autocancellous bone graft mixed with erythropoietin in the bone defect and holes from cortical screws.

DISCUSSION

The main approach to the treatment of bone non-unions in small breed dogs and cats consists in removal of existing implants, necrotic tissue and fragments, surgical debridement of bone ends to bleeding periosteum and endosteum, application of bone grafts or bone substitutes and reliable internal or external fixation [2,3]. In this clinical case, the primary osteosynthesis with plate and screws has failed. The loosening of implants due to the insufficiently restricted movement in the post-operative period has resulted in impaired vascularization and atrophic non-union. The decreased blood supply to the distal radius, specific for small breed dogs, may be another factor with adverse impact on bone regeneration in this region. Locking plates are considered a suitable method for osteosynthesis of radius/ulna fractures in cats and dogs under 2 kg, especially those with small bone fragments [24]. In our patient, the distal radial fragment was short, but a dynamic compression plate was used as a locking plate was not available at the time of first surgery.

The reported great percentage of canine antebrachial non-unions (up to 60% of cases) requires application of a method for bone healing promotion [14]. Bone grafting is a preferred approach, in particular autologous bone grafts that are well integrated in the host bone with minimum risk of infection or displacement [19]. The utilization of autocancellous bone grafts not only ensures the osteoconductive 3-dimensional structure onto which new bone can form, but also provides cytokines and growth factors for bone healing [16]. In dogs, preferred donor sites for cancellous autografts are the proximal humerus, the ilium and the proximal tibia. In this case, the humerus was chosen due to the abundance of cancellous bone, the easy approach and safety [8].

The techniques for bone regeneration stimulation in complicated and non-healing fractures are of increased interest for human and veterinary orthopaedics [1,21]. The autologous cancellous bone graft in the dog was applied together with erythropoietin (EPO) in order to promote bone healing. Previous studies affirmed the indisputable osteoinductive and angiogenic potential of EPO. It stimulated the differentiation of mesenchymal stem cells into osteoblasts [25], thus improving bone regeneration and speeding bone healing [7,15]. Furthermore, EPO modulated inflammation by antagonising pro-inflammatory cytokines [17]. Experimental studies in mice, rats and rabbits have explored the effect from EPO application, either alone or with a bone substitute, on bone regeneration [17,22]. It was histologically proven that the local application of EPO stimulated angiogenesis and potentiated the effect of bone substitute at the bone defect site, probably due to the larger amount of newly formed blood vessels [9]; also, it increased fibrous tissue and blood vessels formation in EPO-treated in comparison to untreated bone defects [23].

After the removal of fixation implants and filling of holes from the screws with autocancellous bone

soaked with erythropoietin, the limb was immobilized with a rigid splint and the patient was kept in a cage for 10 days to restrict movements. Despite the frequent complications from the application of rigid splints in small dog breeds leading to malunion or non-union [26], this method for immobilization was chosen because the plate osteosynthesis has failed.

Monitoring radiography and computed tomography evidenced that the bone defect healed for a short period (12 days). In an earlier study [13], the application of a cancellous bone graft in a dog with complicated fracture of the radius and ulna has resulted in a partial weight bearing with the limb by the end of the 2nd post operative week, with radiological signs of complete engraftment by the 4th week after surgery. In our belief, the more rapid positive outcome in our patient was most probably due to the local application of erythropoietin with the autograft.

In conclusion, augmentation of bone healing requires different and often nonstandard solutions. In the present case of atrophic non-union with possibly avascular bone ends, the osteogenic and angiogenic potential of erythropoietin, mixed with the autologous cancellous bone graft for filling of bone defect and bone holes from cortical screws, resulted in a rapid boosting of bone healing with convincing diagnostic imaging evidence for bone regeneration as early as 12 days after the operative revision. Thus, erythropoietin seems a promising adjunct to the functional therapy for bone non-union regeneration.

MANUFACTURERS

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