Scoring systems used in assessing the effect of intradiscal application of BMP-7 in a canine model of spontaneous intervertebral disc degeneration.

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Introduction

Low back pain is a problem experienced by humans as well as dogs. In severe cases it can result in chronic disability. Intervertebral Disc Degeneration (IVDD) is one of the most common causes of low back pain.[1] IVDD is a consequence of changes in the biochemical and biomechanical environment, resulting in alternation of the matrix metabolism.[2]

The intervertebral disc (IVD) arises from embryonic notochord and mesenchyme and consists of four different structures; the nucleus pulposus (NP), the annulus fibrosus (AF), the transitional zone (TZ) and endplates (EPs). In the center of the IVD lies the NP, a gel like well-rounded bean-shaped structure.[2] The main cell-type in the NP of healthy IVDs is notochordal cells, that are characterized by cytoplasmic vesicles. These cells are found in clusters and produce a matrix that is rich in proteoglycans (PG) and collagen (mainly type 2). PGs are macromolecules, consisting of a protein backbone and GAG-sidechains which are highly negatively loaded. The resulting osmotic gradient attracts lots of water, causing the NP to be hydrated. This is one of the main reasons that the disc can withstand pressure. The NP of an adult animal consists for 20% of PGs and up to 70% in juvenile animals.[3] The NP is surrounded by the AF, a network of fibrous lamellae. The AF consists of collagen (mainly type 1), elastin fibers and fibroblast-like cells.[2, 4] The ventral AF is twice to thrice as thick as the dorsal AF. The transitional area between the NP and AF is the TZ. The EPs are cartilaginous structures, forming the cranial and caudal borders of the IVD. These EPs play an important role in providing the IVD with nutrients (small molecules like oxygen and glucose).[2]

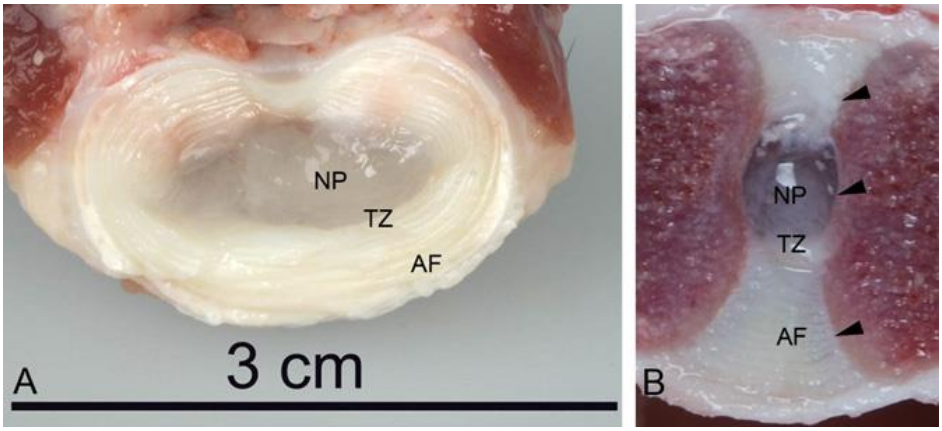


Fig. 1. Transverse (A) and sagittal (B) sections through a L5–L6 intervertebral disc of a mature non-chondrodystrophic dog, showing the nucleus pulposus (NP), transition zone (TZ), annulus fibrosus (AF), and endplates (arrowheads).[2]

In the case of IVDD, the first cellular change is a decrease of the clusters of notochordal cells and are replaced by small clusters of chodrocyt-like cells.[2] These cells produce a matrix similar to hyaline, consisting mainly of disorganized collagen fibers. This process is called chondrification. The decreased concentration of the hydrophilic segments as PGs, results in a

decreased viscosity of the NP. Also a change in the distribution of collagen (increase of type 1) occurs, leading to a failure to form a correct structural framework.[5] The decreased viscosity and size of the NP results in a reduced intradiscal pressure. In order to withstand pressure forces, the AF growths in size but becomes stiffer and weaker. This causes a structural failure which makes the disc less resistant to tensile forces. Combined with a change in force direction, this can cause the AF to buldge or tear after compressive loading, resulting into herniation.[2]

Current therapies focus on alleviating pain and reducing neurological signs and include medication, physical therapy and surgery (e.g. fusion or disc replacement). However, these therapies will not lead to restoration of the disk and recurrence of IVDD is often seen. Furthermore, surgical methods also influence the disc structure, which may eventually lead to further degeneration.[6, 7] Therefore, surgery is often only used in an advanced stage of disease. New therapies focusing on repairing the matrix and structural integrity of the NP and AF, at an early stage of disease, show a lot of potential for a long term restoration. These potential regenerative treatments include cell-based therapies, gene therapy and growth factors therapies.[4]

Several studies have shown that bone morphogenetic proteins (BMPs) have a potential regenerative effect in IVDD.[8, 9] BMPs are part of the transforming growth factor beta (TGFβ) superfamily and are involved in numerous developmental processes including osteogenesis and chondrogenesis. BMP signaling involves type I and type II TGF-β superfamily receptor polypeptides, leading to phosphorylation of SMAD proteins.[8, 10] One of these growth factors that shows a lot of promise for IVD regeneration is BMP-7. BMP-7 increases metabolic activity in bovine and human disc cells in vitro, resulting in proteoglycan accumulation by stimulation of cell proliferation and proteoglycan synthesis.[7, 11] An in vivo study in a rabbit annular-puncture disc degeneration model has shown that an injection with BMP-7 led to restored biomechanical properties of the degenerated disc, 8 weeks post-injection, by anabolic stimulation.[1] The injection with BMP-7 led to an increase of PG content, which consequently caused rehydration of the extracellular matrix, restoring the ability to resist compressive loading. Also an increase in collagen type 2 strengthened the disc structure. 4 weeks after injection of BMP-7, disc height increased significantly compared to the control group. Another study using this model has shown restoration of disc height, 6 weeks after BMP-7 injection, which was retained up to 24 weeks.[6]

Growth factors are known for having a short half live and chemical instability. Controlled release systems are biomaterials like hydrogels or microspheres made of biodegradable polymers in which proteins can be encapsulated and released slowly. The use of these controlled release systems could help maintain a steadier concentration of BMP-7 in the IVD.[12, 13]

Up to this point, no research has been done in an animal model that would be directly comparable to spontaneous human IVDD. In the course of developing future treatments for dogs and humans, the regenerative effect of BMP-7 on the IVD has to be researched in an animal model with spontaneous IVDD at an early stage of degeneration. Dogs can be divided in non-chondrodystrophic and chondrodystrophic breeds. Chondrodystrophic dog breeds have a disturbed endochondral ossification and are also predisposed for IVDD. In these breeds an early onset of degeneration in all intervertebral discs occurs from 1 year of age.[14] Changes in histology, radiographic appearance and biochemistry largely related to pathology in aging human IVDs have also been observed in dog IVDs, especially in chondrodystrophic breeds. In

contrast to most other animals, chondrodystrophic dog breeds, like humans, have a low concentration of notochordal cells.[15] The Beagle, used in this study, is one of these chondrodystrophic breeds and therefore a suitable model.[14]

Aim of the study

This study is the first step towards a preclinical application of BMP-7 in veterinary patients and extrapolation to clinical studies in human patients. The study serves three objectives:

1. To define the dose of BMP-7 resulting into an optimal anabolic effect on spontaneous degenerated lumbar IVD of laboratory Beagles.
2. To evaluate the additive effect of a controlled release system compared to a bolus injection. These results will be used to optimize the loading concentrations for the subsequent in vivo experiment with BMP-7 loaded controlled release systems in dogs.
3. To determine the applicability of T1rho imaging for evaluating regenerative treatments in IVDD and compare this to the more commonly used T2-weighted and T2-mapping imaging currently used for evaluating this condition.

This paper will focus on the different scoring systems used to assess the effect of the treatment with BMP-7 on the IVDs. The scoring systems that we will review are the Pfirrmann score, the Thompson score and the Disc Height Index (DHI).

The Pfirrmann score is the most commonly used grading methods for IVDD, using T2-weighted MR images. This method has been validated for IVDs of dogs by Bergknut *et al.*[16] IVDs can be divided into 5 categories. These categories are based on the loss of signal intensity, the evaluation of structures, disc height and the distinction between NP and AF.[17]

The Thompson score is based on gross pathological changes in macroscopic slices of the IVD and has also been validated for IVDs of dogs by Bergknut *et al.*[18] As visible in figure 3, four structures of the IVD (NP, AF, EPs and the vertebral bodies) are scored, leading to 5 different grades of degeneration.

To assess the effect of BMP-7 on disc height in our study, disc height was measured on T2-weighted MR images and expressed as DHI using the method described by Masuda *et al.*[6] (see figure 4)

Materials & Methods

Animals

In this study, with DEC number: 2012.III.07.065, seven male laboratory Beagles with a median age of 1.3 years (range: 1.1 to 1.8) were enrolled. Prior to surgery, the dogs underwent a general, orthopedic and neurological examination. To determine the degree of IVD degeneration T2- and T1rho weighted images as well as T2 maps were obtained prior to surgery. To be included the dogs needed to show signs of early IVD degeneration on the MR images (Pfirrmann grade 2). To observe changes in the IVDs these images were also obtained at 6, 12 and 24 weeks after surgery.
After 24 weeks the dogs were euthanized and all lumbar IVDs were collected. Half of each IVD was evaluated macroscopically and histologically. Paraffin sections were stained to determine matrix components, e.g. proteoglycans and collagen I, II and X. Cryosections were obtained from the other part and the NP and AF were separated. One quarter was stored at -70°C in a special buffer solution for biochemical analysis, e.g. GAG and DNA. The other quarter was used to perform a qPCR.

Injected materials

At six randomly selected levels of the lumbar spine a volume of 30 ul was injected into the NP, either by using a 26 or a 27 Gauge needle. The injections consisted of a sham, a bolus injection with 2.5, 25, or 250 ug BMP-7, and a controlled release system, loaded with 2.5 ug BMP-7 or unloaded. These injections were administered from T13-L1 through L7-S1 via a surgical procedure. Disc T12T13 served as untreated control in every dog. Also the discs directly cranial and caudal of the disc injected with the highest concentration of BMP-7 were left untreated. This made it possible to examine if an injection with high concentrations of BMP-7 would also have an effect on the adjacent IVDs.

MRI

MRI was performed using a 1.5 Tesla MR scanner using a spine array coil (Philips Healthcare, Best, The Netherlands). Sagittal T2-weighted images were acquired using a turbo-spin echo sequence. The scan was made with the following parameters: FOV 200 mm, slice thickness 2 mm, acquisition matrix 332 x 306, TE/TR = 100ms/2600ms.
The dogs were put under general anesthesia and a laryngeal mask was placed in order to support respiration and maintain a constant level of anesthesia. Monitoring was done by means of an ECG and measuring the levels of O2 and CO2. The dogs were placed on their backs, hind legs towards the machine. The front paws were bound to the cranial side with linen cloths to stabilize the thorax and establish a good passage of the IV infusion. To stabilize the neck and spine and avoid tilting, cushions were placed under the nose, the femurs and the sides of the thorax. The spine was carefully placed in a straight line to obtain a symmetric and correct sagittal image. To avoid an oblique image the sternum had to be positioned directly above the spine.
The dogs were scanned in a fixed order so each dog was scanned at the same time of the day in all four MRIs. This was done in order to avoid the possible influence of the time of the day on the DHI.

Pfirrmann score

The T2-weighted images were used for assessment of degenerative grade of each disc (n = 252) using the system described by Pfirrmann *et al.*[17] The criteria according to Pfirrmann are visible in figure 2. The scoring was performed by three individual observers (one radiologist, one PhD-student and one master-student).

Pfirrmann grade	Structure	Distinction between NP and AF	Signal intensity	Height of IVD
1	Homogeneous and bright white	Clear	Hyperintense and isointense to CSF	Normal
2	Nonhomogeneous with or without horizontal bands	Clear	Hyperintense and isointense to CSF	Normal
3	Nonhomogeneous and gray	Unclear	Intermediate	Normal to slightly decreased
4	Nonhomogeneous and gray to black	Lost	Intermediate to hypointense	Normal to moderately decreased
5	Nonhomogeneous and black	Lost	Hypointense	Collapsed disk space

AF = Annulus fibrosus. NP = Nucleus pulposus.

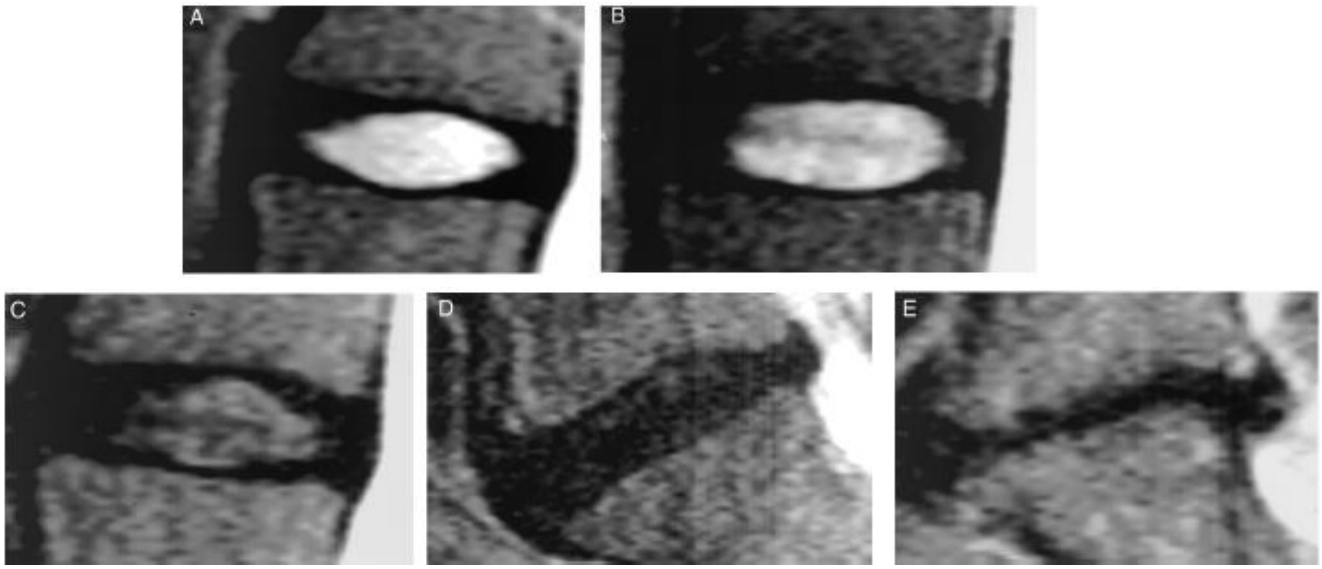


Fig. 2. Grading criteria of Pfirrmann score to classify IVDD in dogs. A-E, a series of midsagittal T2-weighted MRI images of dog IVDs representing Pfirrmann grade 1 to 5.[16, 17]

Thompson score

After the dogs were euthanized, the spine was collected from T12 to S1. All the soft tissue was removed from the spine so the bone could be cut with a mechanical saw. The vertebral bodies were sawn in half from the lateral side so the IVD remained intact with half of the adjacent vertebral bodies attached. Each IVD was then cut in half in craniocaudal direction to obtain longitudinal cross-sections. Macroscopic photos of these cross-sections were used for the Thompson score of each disc (n = 252). The criteria as described by Bergknut *et al.*[18] are visible in figure 3. The scoring was performed by three individual observers (one PhD-student and two master-students).

Thompson grade	Nucleus pulposus	Annulus fibrosus	End plates	Vertebral bodies
1	Bulging gel	Discrete fibrous lamellae	Hyaline; uniform thickness	Rounded margins
2	White fibrous tissue peripherally	Mucinous material between lamellae	Irregular thickness	Pointed margins
3	Consolidated fibrous tissue	Extensive mucinous infiltration; loss of annular-nuclear demarcation	Focal defects in cartilage	Early chondrophytes or osteophytes at margins
4	Horizontal (vertical) clefts parallel to end plate	Focal disruptions	Fibrocartilage extending from subchondral bone; irregularity and focal sclerosis in subchondral bone	Osteophytes < 2 mm
5	Clefts extend through nucleus and annulus		Diffuse sclerosis	Osteophytes > 2 mm

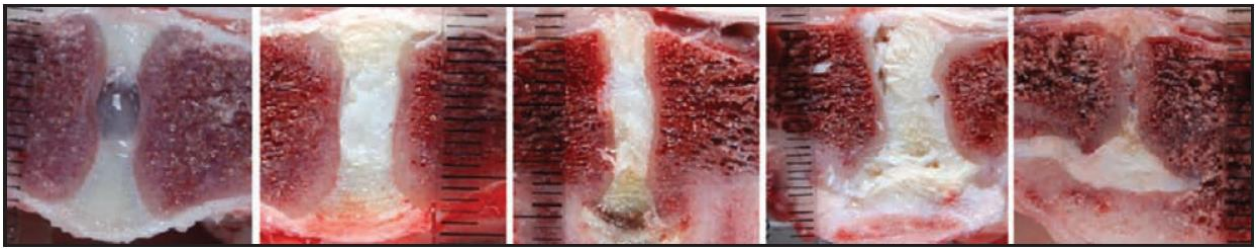
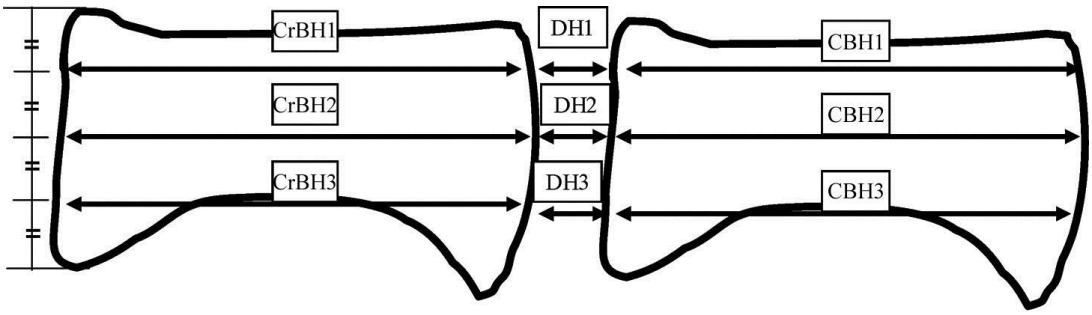


Fig. 3. Grading criteria of Thompson score to classify IVDD in dogs. A series of photographs of midsagittal plane divided dog IVDs representing Thompson grade 1 to 5.[18]

Disc Height Index

The disc height and the vertebral body height of all discs (n = 252) were measured on the T2-weighted MR images using the Philips DICOM Viewer R3.0. The height of the intervertebral disc was subsequently expressed as the disc height index (DHI) using the method of *Masuda et al.*[6] To correct for the individual size of the dog the DHI in calculated by dividing the DH with the length of the adjacent vertebral bodies. The average disc height is calculated by measuring the height in the posterior, middle and anterior portion of the IVD. The lengths of the cranial en caudal vertebral body are measured at the same three levels. (figure 4)



$$DHI = \frac{2(DH1 + DH2 + DH3)}{(CrBH1 + CrBH2 + CrBH3) + (CBH1 + CBH2 + CBH3)}$$

Fig. 4. Method for radiographic measurement of disc height (DH). The intervertebral disc height is expressed as DHI using the method of Masuda *et al.*[6] The average disc height was calculated by measuring the height in the posterior, middle and anterior portion of the IVD and was divided by the average of adjacent cranial vertebral body height (CrBH) and caudal vertebral body height (CBH).

The position of the posterior, middle and anterior part of the vertebral bodies and the disc were determined, using the following procedure:
First, two lines were drawn, parallel to the IVD, at the widest most cranial and caudal portion of the two vertebral bodies adjacent to the IVD. By drawing two lines from the top and bottom point to the middle point of the previous set line, the vertebral bodies were divided in 4 equal parts, allowing a line to be drawn through the anterior, middle and posterior part of both vertebral bodies. The DH was measured by connecting the lines made through each vertebral body. An average DHI was calculated by multiplying the sum of the anterior, middle and posterior heights of the IVD by two, then dividing this with the sum of all heights of the adjacent vertebral bodies. (figure 5)

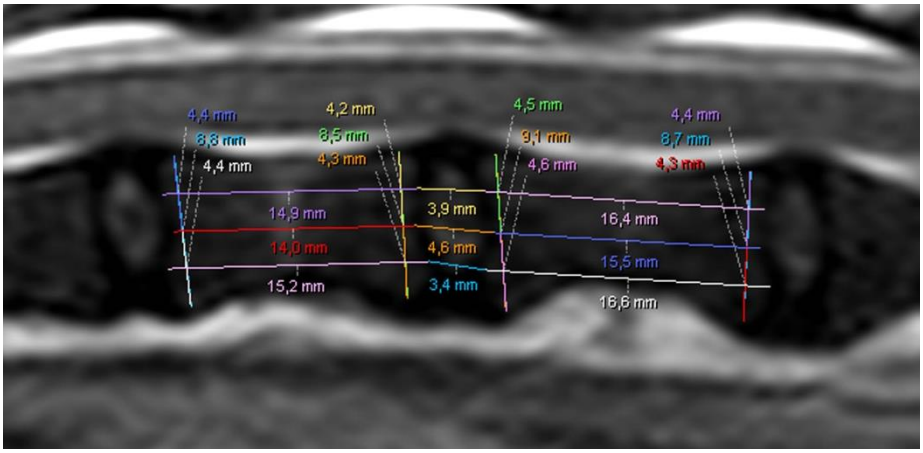


Fig. 5. Method for radiographic measurement of disc height (DH) and adjacent vertebral bodies in Philips DICOM Viewer.

Statistical analysis

The statistical analysis of the DHI was performed with the use of R, version 2.15.2. A linear mixed effect model has been applied. The dog was included as a random effect. The IVD level was included as fixed effect (T12-T13 and L7-S1 have a different size as the other lumbar discs).
The change in DHI in the course of time per injected condition was examined. Also the DHI between injected conditions were analyzed.

Results

Pfirrmann

61 out of the 63 (96.8%) IVDs were assigned a grade 2 in all 4 MRI's. One disc (L7-S1) that was injected with a sham was assigned a grade 2 in the first MRI and a grade 3 in the three following MRIs. The L3-L4 of a different dog that was not injected started with a Pfirrmann score 3 and showed a score 2 in the other three MRIs. No correlation was observed between the injected conditions and the Pfirrmann score.

X-ray and CT:

The X-ray radiography and CT-scans of 2 out of the 7 spines were unremarkable. The CT-scan of one spine showed a symmetric extensive intervertebral disc herniation at the lumbosacral junction. This disc was injected with the highest concentration of BMP-7. Smoothly marginated bridging new bone formation was noted in 4 spines. In all 4 spines the new bone formation was seen ventrally or laterally of the discs injected with 250 ug BMP-7. In one of these spines, minimal new bone formation was also noted in the soft tissue lateral to the cranial endplate of L4. L3-L4 in this spine was injected with 25ug BMP-7.

Thompson

All but one IVD scored a Thompson score 2. Only one disc, that was injected with 250 ug BMP-7, was rated a Thompson score 4. (figure 6) Striking are the irregular end plates and the sclerosis in the subchondral bone. Also visible are clefts in the NP, some disruptions in the AF and osteophyte formation in the vertebral bodies. On the dorsal side of the IVD a gel like material can be seen between the AF and overlaying fat. This material has the same aspect as the NP and could possibly be herniated material.
Furthermore, bone formation ventral to the IVD has been detected at two sites. (figure 7) Both of these discs were injected with the highest concentration of BMP-7.

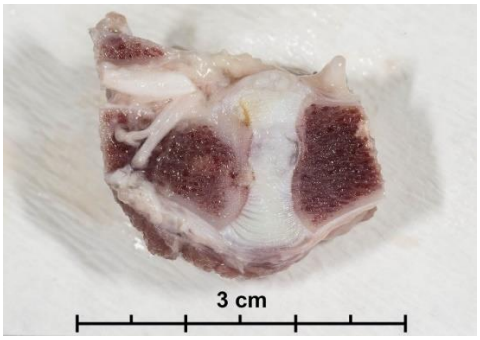


Fig. 6. Photograph of midsagittal plane divided IVD with Thompson score 4.

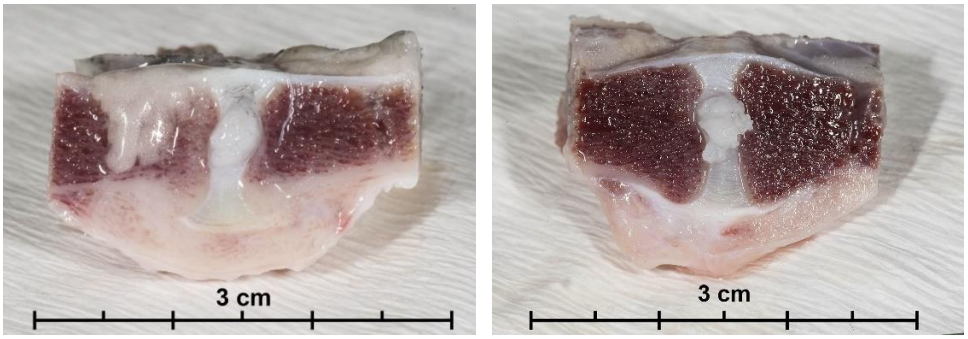


Fig. 7. Photographs of midsagittal plane divided IVDs with bone formation ventral to the IVD.

Disc Height Index

The mean DHI per injection type for every MRI moment is visible in figure 8. No significance changes were observed when comparing the DHI in the four MRI's per injected condition. Also the DHI between injected conditions were analyzed. No significant differences were found.

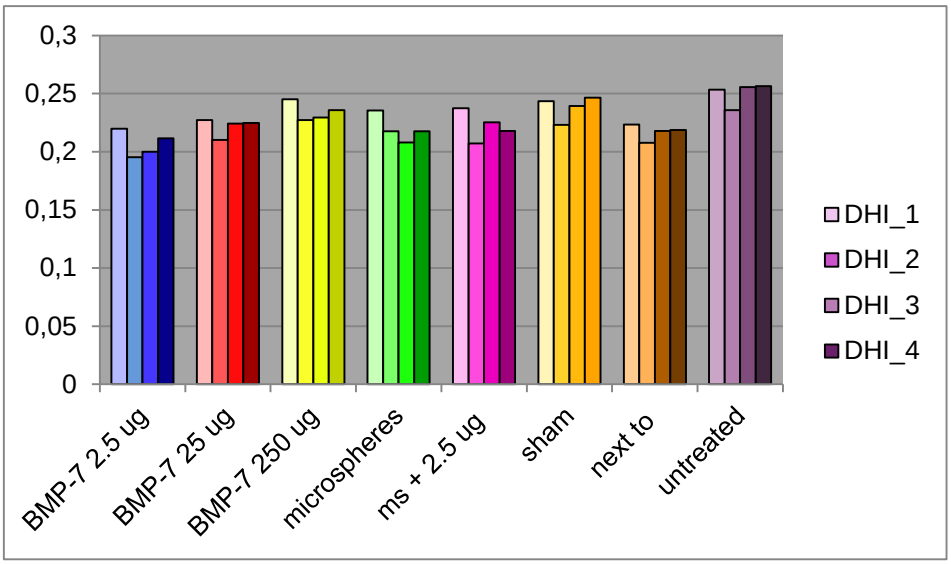


Fig. 8. Mean DHI per injection type for every MRI moment.

Discussion

In this paper we focused on reviewing three different scoring systems (Pfirrmann score, Thompson score and DHI) that were used to assess the effect of BMP-7 injections on IVDs in Beagles with early stage disc degeneration. Firstly, we will discuss the observed effect of BMP-7, secondly we will discuss the scoring systems.

BMP-7

Even though previous research, with different in vivo animal models, showed a regenerative effect of BMP-7, in this study no significant effect of BMP-7 on the IVD was observed in any of the scoring systems. As we are working with a dog model for the first time we must consider which differences, compared to the models with other animal species, could explain these results. The biologic effects of growth factors, like BMP-7, are dependent upon the stage of disease (grade of disc degeneration), a steady and effective concentration of BMP-7 and the cell composition and amount of cells present in the IVD.

Stage of disease

Research in rabbit models showed an increase of PG content in the NP and AF and an increased disc height after injection of BMP-7.[1, 6, 19] In these models either the IVD is punctured to induce a decrease in disc height[1, 6] or the chemonucleolytic agent chondroitinase ABC is administered in the IVD,[19] to simulate a degenerated disc. Thereafter, BMP-7 was administered intradiscally. An increase of disc height and PG content was seen after BMP-7 injection in all three studies. Unfortunately, the degree of disc degeneration before and after puncture or chondroitinase ABC administation is not mentioned in these articles. However, the decrease of DH after puncture ranged between 27% and 34%, corresponding to a Pfirrmann grade of at least 3 and most likely 4.[17]

To assess the possibility of BMP-7 treatment of naturally occurring disc degeneration at an early stage of degeneration, our model used dogs with spontaneous degeneration. These dogs had a Pfirrmann score of 2 and therefore, had lower grades of degeneration than in the induced models of IVD degeneration. This difference in degeneration grade at the moment of injection could explain the lack of observable effect of BMP-7 in our study.

BMP-7 concentration

In order for BMP-7 to have a biologic effect, a certain constant concentration needs to be present in the IVD. The three studies discussed above, where an increased disc height was observed, all used a dose of 100 ug BMP-7. The current study used three different doses, 2.5, 25 and 250 ug BMP-7. This study was performed on dogs instead of rabbits. Therefore, the dogs greater body weight needed to be taken into consideration and a higher concentration of BMP-7 should be necessary for a comparable biological effect. However, these concentrations might still not have been correct as no effect on the disc height could be observed. Several other factors could result in an ineffective concentration of BMP-7 after a bolus injection. Firstly, degradation of BMP-7 could lead to a low concentration. Secondly, BMP-7 might not

stay in place but diffuse out of the IVD. There is evidence that there can be cell leakage after intradiscal injection of stem cells in an induced degeneration animal model with rabbits.[20] The same type of leakage could have occurred in the current study. Certain cell carrier systems or annulus-sealing technologies might prevent the migration of cells out of the IVD. Barczewska *et al.*[21] develop a cell delivery technique using stem cells embedded within hydrogel. No leakage was observed after injection into swine lumber IVD.

Also, the presence of a high concentration of BMP-7 could stimulate certain pathways through which antagonist like noggin and gremlin could be produced, eventually inhibiting BMP-7.[8, 10, 22, 23]

A controlled release system, as used in this study, could help maintain a more steady concentration of BMP-7. Even though fast degradation, diffusion or inhibition could still take place, a correct dose could lead to a constant effective concentration of BMP-7. In this study however, no advantages of the controlled release system compared to the bolus injections have been observed. The maximum possible loading concentration of the used system was 2.5 ug. This concentration might have been too low for an observable effect.

Cells in the IVD

A study performed by An *et al.*[24] studied the effect of intradiscal BMP-7 injection in healthy rabbits with uninjured IVDs. As a result they saw a significant increase in disc height and PG content, compared to the control discs. This shows that the lack of results in our study can't be explained only by the difference in degeneration grade. As previously mentioned notochordal cells, present in the IVD, produce matrix rich in PG and collagen type 2 and might play an important role in regeneration in the IVD. An explanation for the lack of change in disc height in our study compared to previously mentioned rabbit-models,[1, 6, 19, 24] could lie in the fact that chondrodystrofic dogs, in contrast to rabbits, have no to very limited amounts of notochordal cells.[14]

BMP-7 influences the IVD homeostasis by either anabolic stimulation or catabolic inhibition of cells present in the IVD, resulting in cell proliferation and matrix synthesis.[7, 11] The degenerated IVD has a limited amount of cells which might not be sufficient to stimulate a regenerative effect. Combining growth factor therapies with other therapies, for example cell therapy, might lead to more promising results.

One or all of the factors mentioned above could explain the lack of regenerative effect in this study. Moreover, our data suggests that BMP-7 might have a negative biological effect. Up till now, no negative biological activity of BMP-7 has been observed in any animal model, and was therefore presumed safe to use. In this study, smoothly marginated bridging new bone formation was noted in the spines of 4 dogs. In all 4 spines the new bone formation was seen ventrally or laterally of the discs injected with 250 ug BMP-7. Also, minimal new bone formation was noted lateral in one disc that was injected with 25 ug BMP-7. The question remains if the BMP-7 was correctly injected into the disc or if it was injected just outside of

the disc. Even if the injection was correctly placed into the center of the disc, leakage of injected material outside of the disc can't be excluded. Peripheral tears in the AF (annular tears) have been shown to play an important role in the pathophysiology of IVDD and were demonstrated during the early stages of the disease.[25, 26] It is therefore possible that injected material leaks out of the IVD through these annular tears, causing the ventral or lateral bone formation. This would suggest that BMP-7, in a high enough concentration, is biologically active in the dog. The osteoinductive effect of BMP-7 has been described in previous studies.[27, 28]

Altogether, compared to models with other animal species a difference in the stage of disease, steady and effective concentration of BMP-7 and the cell composition and amount of cells present in the IVD could explain the lack of regenerative effect seen in this study. Furthermore, negative biological activity in the form of an osteoinductive effect can be expected after injecting high concentrations of BMP-7 in the IVD.

Scoring systems

Three different scoring systems (DHI, Thompson score and Pfirrmann score) were employed in the current study. The advantages and limitations of these systems are discussed below.

DHI

The DHI is a quantitative scale with values that can easily be compared between animals as the DHI is corrected for the individual variation in size of the animal. However, to be able to compare these values correctly between animals, we need to be sure that the DH in an individual animal doesn't change during the day. Previous research[29, 30] looked at diurnal changes in human IVDs. Using different ROI, Ludescher *et al.*[30] measured intensity in T2-weighted images in the middle of the disc (the NP), the outer layer of the disc (the AF) and a region in between (the intermediate area). They found a diurnal decrease of intensity in the NP and intermediate areas and an increase of intensity in the AF. This would support their hypothesis that water is pressed out of the NP after a diurnal load cycle, moving to the outer layers of the disc. This pressure would also cause a decrease in disc height at the end of the day.

To avoid influence of diurnal changes in DHI, we used the same schedule and order at every MRI moment in our study. In order to see if the same effect can be observed in dogs, we compared the DHI of T12-T13 between our dogs. This disc was an uninjected control disc in all dogs. If the same diurnal effect is present in dogs, a lower DHI would be expected in the animals that were scanned at the end of the day. However, no significant change in DHI could be observed. This difference in diurnal effect in dog and human could be due to the anatomical variation between both species. The spine in a dog is horizontally positioned where the human spine is vertically positioned, resulting in a different gravitational pull. On the other hand, the same reason could have explained a bigger diurnal effect in dogs. In order to maintain stability in a horizontal positioned spine, more force and traction from muscles is

necessary compared to a human spine, resulting in higher axial compression stress.[31] However, apparently this doesn't play a significant role as no diurnal change in DHI has been observed.

Thompson score

The effect of using the Thompson score is limited by its grading criteria. Many changes in the disc influence the scoring as the criteria include the NP, AF, EPs and vertebral bodies.[18] However, other clear macroscopic changes like bone formation, observed in two of our discs, do not influence the grade of the disc. The Thompson score is still a very valuable scoring system in research settings as it grades macroscopic changes that can't always be seen with diagnostic imaging. Though, this system will not be relevant in a clinical setting as it needs macroscopic sections of the IVD.

Pfirrmann score

Ideally, a classification system for IVDD would be quantitative, minimally invasive, evades observer bias, would be sensitive for early degenerative changes and would correlate with clinical symptoms. The Pfirrmann scoring, now commonly used for clinical assessment of IVDD, is based on T2-weighted MRI and only consists of 5 grades.[16] This classification system works very well in detecting more advanced degenerated discs, but isn't sensitive enough to detect early degeneration. Furthermore, integer-based classification systems are at risk of observer bias and aren't quantitative but qualitative.

Future scoring system

The development of such a quantitative, noninvasive and objective classification system might be possible using T1rho weighted MR images. Research on IVDD using T1rho MRI has shown a lot of potential. First of all, T1rho MRI has been correlated with PG content in vitro.[32, 33] The loss of PG is characteristic for the pathophysiology of early degenerated discs. This would mean that T1rho MRI would be able to detect changes in the IVD at an early stage of degeneration. Several researches have shown a correlation between T1rho and Pfirrmann grading[3, 34-36] and furthermore a correlation between T1rho and clinical symptoms were observed.[35-37] As you can see in figure 9 from Zobel *et al.*[3] T1rho values decrease with higher Pfirrmann scores, as lower water content in more degenerated discs results in lower T1rho values. The range in T1rho values of Pfirrmann score 1 and 2 are very large. This confirms the idea that T1rho is more sensitive for early degenerative changes in the IVD. Therefore, T1rho shows a very large potential in developing a new quantitative scale for IVDD assessment.

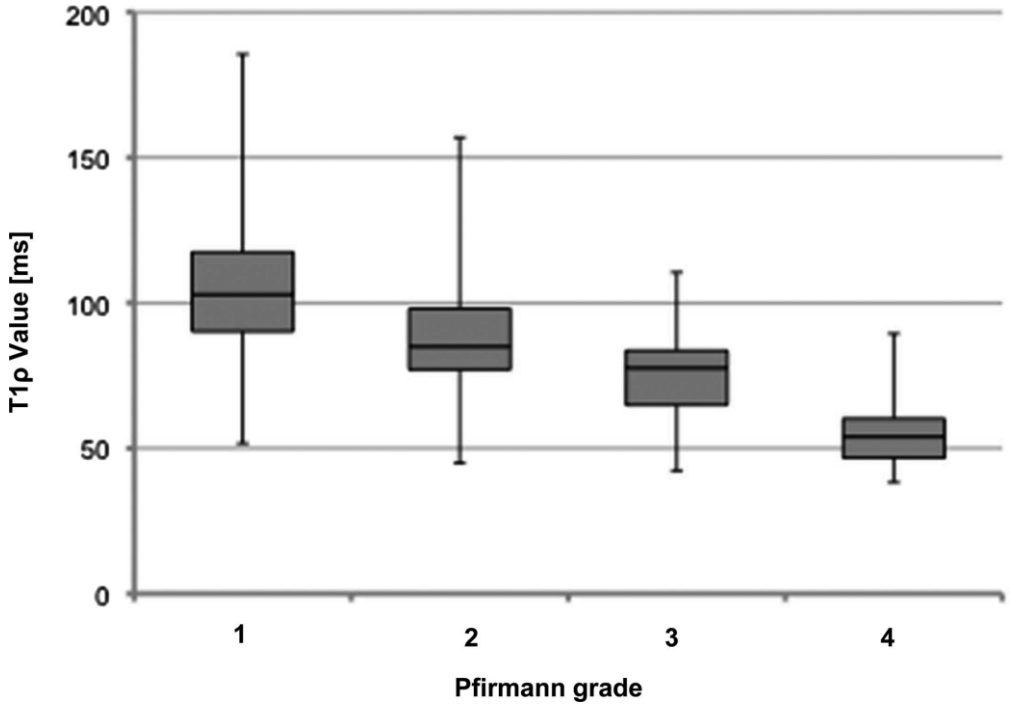


Fig. 9. Box plots of T1ρ value of nucleus pulposus according to the grade of Pfirmann. The boxes represent the median and the interquartile range, with the vertical lines showing the range.[3]

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Appendix:

Table of treatment and results of Pfirrmann score, Thompson score and radiological observations per disc of all Beagles used in this study.

Dog	Disc	Treatment	Pfirrmann	Thompson	Radiology
1	T12-T13	no	2,2,2,2	2	
	T13-L1	BMP-7 25 ug	2,2,2,2	2	
	L1-L2	BMP-7 2.5 ug	2,2,2,2	2	
	L2-L3	no	2,2,2,2	2	
	L3-L4	BMP-7 250 ug	2,2,2,2	2	
	L4-L5	no	2,2,2,2	2	
	L5-L6	mps + BMP-7	2,2,2,2	2	
	L6-L7	mps empty	2,2,2,2	2	
	L7-S1	sham	2,3,3,3	2	
2	T12-T13	no	2,2,2,2	2	
	T13-L1	mps empty	2,2,2,2	2	
	L1-L2	BMP-7 25 ug	2,2,2,2	2	
	L2-L3	sham	2,2,2,2	2	
	L3-L4	no	3,2,2,2	2	
	L4-L5	mps + BMP-7	2,2,2,2	2	
	L5-L6	BMP-7 2.5 ug	2,2,2,2	2	
	L6-L7	no	2,2,2,2	2	
	L7-S1	BMP-7 250 ug	2,2,2,2	4*	IVD herniation
3	T12-T13	no	2,2,2,2	2	
	T13-L1	mps + BMP-7	2,2,2,2	2	
	L1-L2	sham	2,2,2,2	2	
	L2-L3	mps empty	2,2,2,2	2	
	L3-L4	no	2,2,2,2	2	
	L4-L5	BMP-7 250 ug	2,2,2,2	2	new bone formation
	L5-L6	no	2,2,2,2	2	
	L6-L7	BMP-7 2.5 ug	2,2,2,2	2	
	L7-S1	BMP-7 25 ug	2,2,2,2	2	
4	T12-T13	no	2,2,2,2	2	
	T13-L1	sham	2,2,2,2	2	
	L1-L2	mps + BMP-7	2,2,2,2	2	
	L2-L3	BMP-7 25 ug	2,2,2,2	2	
	L3-L4	BMP-7 2.5 ug	2,2,2,2	2	
	L4-L5	mps empty	2,2,2,2	2	
	L5-L6	no	2,2,2,2	2	
	L6-L7	BMP-7 250 ug	2,2,2,2	2**	new bone formation
	L7-S1	no	2,2,2,2	2	
5	T12-T13	no	2,2,2,2	2	
	T13-L1	BMP-7 250 ug	2,2,2,2	2	new bone formation
	L1-L2	no	2,2,2,2	2	
	L2-L3	BMP-7 2.5 ug	2,2,2,2	2	

	L3-L4	BMP-7 25 ug	2,2,2,2	2	minimal new bone formation
	L4-L5	sham	2,2,2,2	2	
	L5-L6	mps empty	2,2,2,2	2	
	L6-L7	mps + BMP-7	2,2,2,2	2	
	L7-S1	no	2,2,2,2	2	
6	T12-T13	no	2,2,2,2	2	
	T13-L1	no	2,2,2,2	2	
	L1-L2	BMP-7 250 ug	2,2,2,2	2	
	L2-L3	no	2,2,2,2	2	
	L3-L4	mps empty	2,2,2,2	2	
	L4-L5	BMP-7 2.5 ug	2,2,2,2	2	
	L5-L6	BMP-7 25 ug	2,2,2,2	2	
	L6-L7	sham	2,2,2,2	2	
	L7-S1	mps + BMP-7	2,2,2,2	2	
7	T12-T13	no	2,2,2,2	2	
	T13-L1	BMP-7 2.5 ug	2,2,2,2	2	
	L1-L2	no	2,2,2,2	2	
	L2-L3	BMP-7 250 ug	2,2,2,2	2**	new bone formation
	L3-L4	no	2,2,2,2	2	
	L4-L5	mps + BMP-7	2,2,2,2	2	
	L5-L6	sham	2,2,2,2	2	
	L6-L7	BMP-7 25 ug	2,2,2,2	2	
	L7-S1	mps empty	2,2,2,2	2	