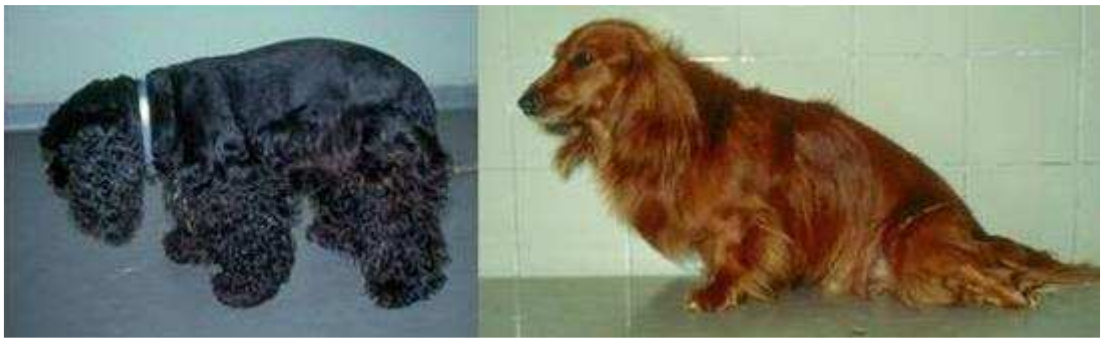
Canine intervertebral disc disease: a comparative study on clinical signs, Pfirrmann MRI scores and histology in surgically treated patients



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Summary

In this retrospective study, data from 74 canine patients surgically treated for intervertebral disc herniations at the University Clinic for Companion Animals in Utrecht was evaluated. The occurrence and spinal location of type 1 and type 2 herniations in the research population, divided in chondrodystrophic (CD) and non-chondrodystrophic (NCD) breeds, was recorded. And most importantly, correlation analysis was performed on the stage of degeneration by using the grading system by Scott and McKee for neurological scores, the Pfirrmann grading system on Magnetic Resonance (MR) images, and an adjusted version of the histological scoring system by Boos et al. on surgically biopsies.

Type 1 herniations had a significantly higher rate of occurrence in CD dogs compared to NCD dogs. Type 1 herniations were more common in the cervical and thoracolumbar spinal areas and type 2 herniations were found to be more common in the lumbosacral segment. No significant age difference was found between the mean ages at which dogs with either type 1 or type 2 herniations were referred to the clinic as well as between the mean ages of CD and NCD dogs.

In both the Pfirrmann and the histological scoring system the scores represent the amount of disc degeneration and they did not correlate significantly with clinical symptoms. The amount of degeneration seen on MRI however does correlate with the amount of degeneration seen in the histological biopsies.

In conclusion, the Pfirrmann MRI scores is significantly related to histology. MR imaging is moreover an useful imaging technique to determine the herniation localization. Future research could focus on whether MRI and histological scores can be used to predict the prognosis of IVD herniation patients.

Moreover; the used population of surgical patients has many similar characteristics when compared to earlier reported characteristics of the canine population at large.

Introduction

In veterinary medicine, intervertebral disc disease (IVDD) is a relatively common condition and as a serious and painful disease it poses a great challenge to veterinarians. Degenerative disc changes occur frequently and become clinically significant when they lead to IVDD. A major consequence of this progressive disorder is an intervertebral disc (IVD) herniation which can cause a multitude of neurological symptoms as a result of degenerate disc material pressuring the spinal cord. The prevalence of IVD herniations in dogs differs per breed but the overall incidence has been reported to be about 2% [5,6]. In human medicine prevalence data show that up till 25% to 30% of the Dutch adult population suffers from radiating leg pain caused by lumbar IVDD. As a consequence about 200.000 medical consultations are rendered to general practitioners clinics every year [20].

Intervertebral disc anatomy & function

All canine vertebrae are joined by intervertebral discs, with the exception of the atlanto-axial joint and the fused sacral vertebrae [2,5,43].

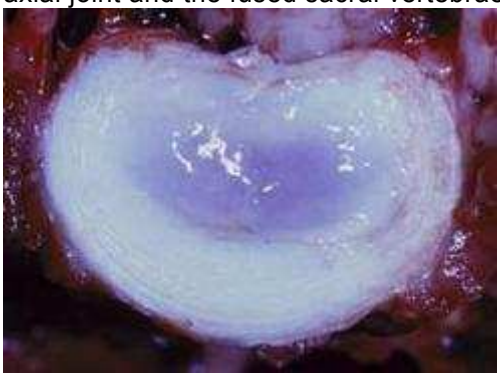

Figure 1: A transverse section of a healthy IVD [7].

Viewed in axial direction the disc is oval in outline, with the longest diameter in transverse (figure 1) [43]. Each IVD exists of a tough outer ring (the annulus fibrosus), a pulpy center (the nucleus pulposus) and two axial cartilaginous end plates (EP) connecting the disc to the adjacent vertebral bodies. Healthy IVDs distribute pressures and transmit stresses and strains over the spinal column, yet being tough and flexible at the same time. In both humans and dogs IVDs make an important contribution to the integrity and functionality of the spine, allowing for everyday movements [5,12]. The annulus fibrosus (AF) is the fibrous ring in which the nucleus pulposus (NP) lies embedded, it is a dehydrated structure composed out of dry weight for almost 70% [5]. The main matrix molecule in the AF is type one collagen [8,28], which is produced by fibrocytes located between the collagen bundles [5,46]. Fibrocytes have an elongated appearance and can also be found in the EPs [35]. In a macroscopic cross-section, the AF appears to consist of concentric rings [5]. Microscopically the AF is seen as separate layers of fibro cartilaginous lamellae, which are each composed of parallel fibrous bundles [22,23]. The NP is an avascular, aneural gelatinous remnant of the endodermic notochord and it occupies a slightly eccentric position, since the ventral and lateral aspects of the AF are thicker than the dorsal aspect [10,12,13,28,35]. The consistency of the NP is semi-fluid in young healthy IVDs and it is kept under continuous pressure by the surrounding AF. The main component of the NP is water, which is attracted by proteoglycan (predominantly aggrecan) [10], during aging the water content is reduced. The NP is composed of loosely organized type two collagen fibrils [10]. Two EPs, consisting of hyaline cartilage, are located cranial and caudal of each intervertebral disc and are in contact with the associated vertebrae [13,18]. The thin central portion of the EPs is permeable for nutrition, which is supplied by capillaries from the vertebral body [11]. Fibers from the AF are anchored with those from the EPs forming strong attachments [22]. The cartilaginous EPs in turn lock into the vertebral bodies via calcified cartilage [35].

Intervertebral disc disease

IVDD is a condition of degenerative origin of the IVD which can lead to a herniation of disc material. Degeneration of the IVD is a process that also occurs during aging. Differentiating pathological IVDD leading to clinical signs from degeneration due to the normal aging process can be challenging [4,6,35]. In the present report IVDD is used for intervertebral disc disease, which means that clinical symptoms have arisen as a consequence of the degenerative disc changes that have occurred in the past. In the progressive process of IVD degeneration – amongst other changes – a shift in cell type can be seen, matrix molecules change and NP' water content falls, leading to a diminishment of the disc's shock absorbing capabilities [19]. Other degenerative changes that deserve mentioning are increased chondrocyte proliferation, increased cell death and gross matrix changes [36]. Furthermore other spinal structures such as the articular facets, vertebral bodies, ligaments and musculature can become secondarily involved in the disease process, worsening the clinical state of the patient. One of the most important consequences of the degenerative process is NP material bursting through the AF and becoming entrapped (bulging or protrusion) whilst compressing neuronal tissue [6,18]. Hansen (1953) was one of the first veterinary researchers to publish about IVD herniations in dogs. In his research he divided IVD herniations into two main types; type 1 and type 2. Type 1 herniations were described to be of considerable size, all layers of the AF were ruptured, and the inner layers of the AF and the NP were occasionally calcified. Type 2 IVD herniations were found to be smaller sized, to have a partially ruptured AF, and to be degenerated in other ways than via calcification [18].

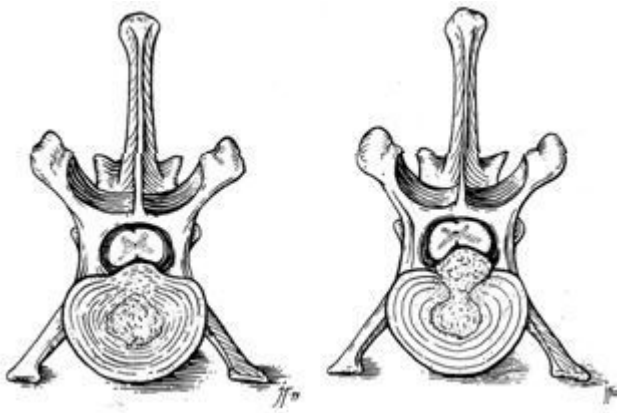

Figure 2: Transverse sections of two intervertebral discs. The left figure shows a type 2 herniation, and the right figure a type 1 herniation [44a].

Clinical signs are largely dependent upon the localization of the IVD herniation, no pathognomonic clinical feature exists [14]. Variables affecting clinical symptoms are the acuteness of onset of the IVD herniation, the impact velocity on the spinal cord, and the mass of possibly extruded material [43]. In cervical IVD herniations 15% to 61% of patients display symptoms of neck pain, guarding of the neck, and muscle fasciculations without neurological deficits [29]. Nerve root signature, i.e. pain apparent on palpation or traction of the limb, caused by lower cervical nerve root compression could be found in 15% to 50% of cervical IVD herniation patients [29], and ataxia, tetraparesis, or paralysis has been reported in 9,1% to 17,6% of patients undergoing surgery [38]. Thoracolumbar IVD herniations may also cause a range of different symptoms, from back pain to severe neurological deficits, such as paralysis with loss of deep pain perception [27]. Clinical signs in lumbosacral patients differ from other spinal locations; pelvic limb lameness, caudal lumbar pain and pain evoked by lumbosacral pressure were the most frequent clinical findings [46], urinary and fecal incontinence can also occur [44b].

Chondrodystrophic vs. non-chondrodystrophic dog breeds

In chondrodystrophic (CD) dog breeds growth plates are calcified sooner in life than in nonchondrodystrophic (NCD) breeds, causing short-limbedness or disproportional dwarfism [30]. Examples of CD breeds are – amongst many others – the Dachshund, Pekinese and Beagle. In IVDs of CD dogs degeneration occurs much sooner than in NCD dogs. Degenerative changes – which start in the NP – have been described in CD dogs as young as two months of age [6]. At an age of one year the NP is already more chondroid of nature than mucoid and at four years of age a certain amount of chondroid metaplasia is always present in the NP of CD breeds [3]. Among the CD breeds the Dachshund is especially predisposed for developing IVDD with a breed prevalence of around 19% [34,37]. In the IVDs of newborn pups notochordal cells are present in the NP

[21]. However in CD dogs these cells disappear early in life [10], while they remain into adulthood in NCD dogs. Notochordal cells may be critical in the maintenance of the IVD, and loss of these cells is a possible factor of importance in the process of disc degeneration in both dogs and humans [21].

Magnetic Resonance Imaging in IVDD
Magnetic Resonance Imaging (MRI) has been accepted as a reliable and non-invasive method of diagnosing spinal disease, including IVD herniations, in dogs [2,26,33,44c, 45]. IVD degeneration and the anatomical disc structure can be clearly visualized by means of MRI, especially in T2 weighted (T2W) sagittal and transverse images. The extent and location of spinal cord compression can readily be determined by looking at – amongst other things – signal intensity. On a T2W MRI, a healthy NP has a hyperintense signal when compared to the AF and it is isointense to liquor [2]. Axial MR images provide information about the location of the herniated material. Knowledge of the location of the lesion must be available before surgical treatment is considered an option [14]. The basic appearance of the canine spine is as follows; the gray scale from brightest to darkest on T1 weighted images of the human spine and related structures ranges as follows: from fat being the brightest, marrow cavity and cancellous bone, nucleus pulposus, spinal cord, muscle, liquor, annulus fibrosus and ligaments to cortical bone being the darkest. T2W images have a similar gray scale, except for the liquor and NP since they are less intense than fat, but more intense than the remaining structures [26].

Treatment options
Treatment may be conservative or surgical. Conservative, non-surgical, treatment is considered to be applicable for mild IVD herniation patients where pain is the only clinical sign. In general it consists of cage rest for 2 to 8 weeks and is often combined with medication [25]. Since the AF is an avascular structure the animal must remain calm for weeks on end in order for sufficient healing to occur [11,44d]. Surgical treatment is indicated in patients with severe pain and/or neurological deficits or in case conservative treatment fails [44d]. IVD herniations can be best treated by means of decompression as it provides the most rapid resolution of clinical signs. Laminectomy allows removal of the herniated degenerated disc material from the spinal canal, though it is a technically difficult surgery to perform [44d]. In order to apply this technique safely, accurate data of the lesion localization must be available and the surgery must be performed by a specialized surgeon to have the highest change of success [3].

To conclude this introduction, a multitude of research and literature about the clinical signs, diagnostics and histology of IVDD is currently available. However the relationship between these three factors has not yet been described. In this research project the focus lies on the rate of occurrence of IVD herniations in different groups, and most importantly on the comparison of neurological, MRI, and histological scores in surgically acquired IVD material. During the scoring of histological material the conclusions drawn by Hansen (1951) [18] were kept in mind by the author, in order to make a thoughtful comparison with the results of this research project.

Research aims
The research aims of this project were:

- To determine the ratio and spinal distribution of type 1 and type 2 IVD herniations in a surgical treated group of chondrodystrophic and non chondrodystrophic dogs.

- To investigate differences in the age at surgery between chondrodystrophic and nonchondrodystrophic dog breeds with IVDD, and between dogs with type 1 and type 2 IVD herniations.

- And most importantly, to determine the correlations between clinical neurological scores, Pfirrmann MRI grading and histological biopsy scores in a population of outpatient dogs.

Materials and Methods

Dogs suspected of IVDD referred to the University Clinic for Companion Animals of the Utrecht University from March 2008 until September 2010 were clinically assessed by veterinary students and veterinarians and diagnostic imaging (MRI) was performed. After conformation of the diagnosis and localization of the IVD herniation, all patients underwent surgical treatment. A total of 74 dogs of which a MRI and surgical biopsies were available were included in this research project.

Statistics
Patient data was analyzed using SPSS (version 16.0) and Winepiscope (version 2.0). After testing for normality (using a Shapiro-Wilk W test) a two independent samples T test was ran to compare the ages of different patient groups; an odds ratio was calculated for the rate of occurrence of type 1 and type 2 herniations in CD and NCD dogs; relative risks were calculated on the proportions of IVD herniations in the different spinal areas in CD and NCD dogs and for the two types of herniations. Moreover, Cohen's weighted kappa was used to determine intra-observer reliability for the two separate rounds of histological and Pfirrmann MRI scoring. For the histological scoring kappas were determined for the separate categories, since the total histological scores had to many possible outcomes. The intra-observer reliability between the two histological scoring rounds was determined by using Spearman's rho correlation coefficient.
According to Landis and Koch (1977) the kappa agreement (κ) was rated as follows: κ0-0.20 indicated slight agreement, κ0.21-0.40 fair agreement, κ0.41-0.60 moderate agreement, κ0.61-0.80 substantial agreement, and κ0.81-1.00 excellent agreement [24]. In the correlation analysis between Pfirrmann MRI, histological and clinical neurological scores Spearman's rho correlation coefficient was determined. Because the neurological, Pfirrmann and histological scores were not normally distributed, Spearman's rho instead of Pearson's correlation coefficient was used. The outcome of the spearman's correlation coefficient is always between -1 and +1, in which -1 and +1 indicates a perfect agreement between the variables. A correlation of zero means there is no relationship between them. When there is a positive correlation between two variables, as the value of one variable increases, the value of the other variable also increases. The significance means whether or not the found correlation is different than zero. As a rule of thumb, correlation coefficients between .00 and .30 are considered weak, those between .30 and .70 are moderate and coefficients between .70 and 1.00 are considered high.

Neurological grading
From the clinics patient data system (Vetware) breed, age, and clinical data were collected for 74 patients. Dr. B.P. Meij helped in interpreting data from the clinical charts into clinical neurological scores, based upon the scoring system by Scott and McKee (1997,1999) [40,41], as depicted in table 2.

Table 2: The neurological grading system, according to Scott and McKee (1997 and 1999) [40,41].

Neurological Grade	Description
0	No abnormalities
1	Pain only
2	Paraparesis, ambulatory
3	Paraparesis, non ambulatory
4	Paralysis, deep pain perception present, with or without loss of bladder control
5	Paralysis, with loss of both deep pain perception and bladder control

Magnetic resonance imaging
Prior to surgery every patient enclosed in this study underwent a MRI scan; 70 patients at the Utrecht University small animal clinic, and three patients at the Veterinary MRI Centre in Dordrecht. The MRI scanner used in Utrecht was a 0.2 Tesla open magnet (Magnetom

Open Viva, Siemens AG, Germany) with a 16 cm-diameter multipurpose flex coil. Repetition times of 3,75 to 4,45 milliseconds (ms) and echo times of 117 ms were used. The MRI scanner used in Dordrecht had a 0.23 Tesla magnet with repetition times of 3,00 to 3,40 ms and echo times of 90 ms. All MRI's were uploaded and graded in the Web1000 5.1 system (Clinical Review Station, Agfa-Gevaert N.V.), on a HP1702 monitor.

Histological material
Laminectomy was performed from different surgical approaches and in each approach different parts of the herniated IVD were collected (table 1) The surgical approach was dependent on MRI data and lesion localization. IVD material from 44 patients was used for histological scoring, since in 44 patients both AF and NP material was available. After fixation in formalin all discs were embedded into paraffin, sliced with a microtome and subsequently stained with H&E and Picrosirius red / Alcian blue.

Table 1: Overview of the different surgical approaches for each spinal area and breed group and the intervertebral disc material generally collected in that location. (AF = annulus fibrosus, NP = nucleus pulposus, CD= chondrodystrophic dog breeds. NCD= non-chondrodystrophic dog breeds)

Hernia localization	Breed group	Surgical approach	Material generally acquired
Cervical	NCD	Ventral	AF
Cervical	CD	Ventral	AF + NP
Thoracolumbar	CD + NCD	Lateral	AF + rarely NP
Lumbosacral	CD + NCD	Dorsal	Dorsal AF + chaotic NP

Haematoxylin stains acidic structures purplish blue and eosin stains basic structures red or pink. This leads to blue stained nuclei and a pink or red cytoplasm. Picrosirius red / Alcian blue stains collagen red, sulfated and carboxylated acid mucosubstances (proteoglycans) blue, nuclei blue-black and other tissue elements yellow [17]. All slides were scored using a standard optical microscope (Leica DM LS2), with 5x, 10x, 40x and 100x lenses.

Herniation type classification
On MRI, the herniation was evaluated by the surgeon as a possible type 1 or type 2. This diagnosis was confirmed during surgery. In a type 1 IVD herniation NP material can be found within the vertebral canal. The herniated NP material can have a range of possible appearances – from white, paste like to a mixture with clotted blood – depending (amongst other things) on the acuteness of onset of the herniation and the duration of it being in situ. In type 2 herniations the AF bulges and impinges on the spinal canal.

MRI scoring according to Pfirrmann
T2 weighted sagittal MR images were assessed according to the classification system by Pfirrmann et al. [33]. This scoring system was validated for use in dogs by Bergknut et al. 2010 (in press). Bergknut also supervised the author on the use of this scoring system. After some practice the author scored all MR images. Later, the MR images were scored again, to be able to determine intra-observer reliability. Only the surgically treated IVD was scored. Determination of the affected intervertebral disc was aided by reading radiologists MRI reports. In one patient it was not possible to determine and score the affected disc because of birth anomalies (lordosis, scoliosis, spina bifida and transitional vertebrae) therefore this patient was excluded from the correlation analysis. The Pfirrmann scoring system, used in 73 dogs, is depicted in table 2. Examples of the different scores can be seen in figure 3, 4, and 5.

Table 2: The MRI scoring system according to Pfirrmann [33].

Grade	Structure	Distinction of Nucleus and Annulus	Signal intensity	Height of Intervertebral Disc
I	Homogeneous, bright white	Clear	Hyperintense, isointense to cerebrospinal fluid	Normal
II	Inhomogeneous, with of without horizontal bands	Clear	Hyperintense, isointense to cerebrospinal fluid	Normal
III	Inhomogeneous, gray	Unclear	Intermediate	Normal to slightly decreased
IV	Inhomogeneous, gray to black	Lost	Intermediate to hypointense	Normal to moderately decreased
V	Inhomogeneous, black	Lost	Hypointense	Collapsed disc space

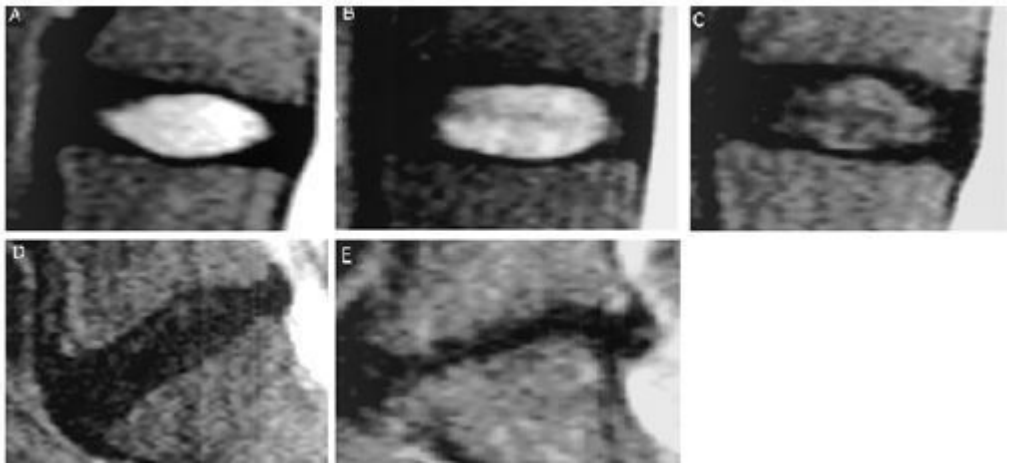

Figure 3: Pfirrmann MRI grades 1 (A) – 5 (E) from the top left corner to the lower right corner of human IVDs [33].

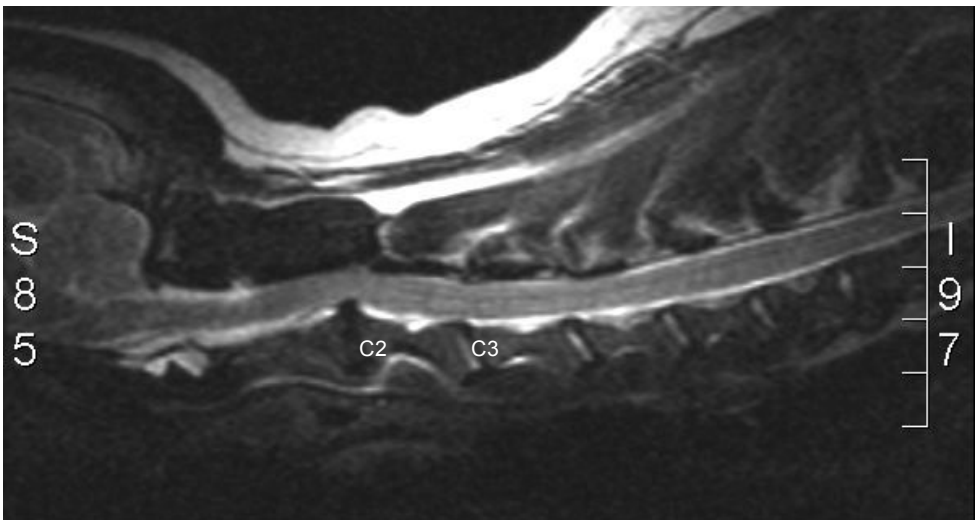

Figure 4: AT2 weighted MRI of the cervical spine of an English Cocker Spaniel with an IVD herniation between C2 and C3 with a Pfirrmann score of 4.

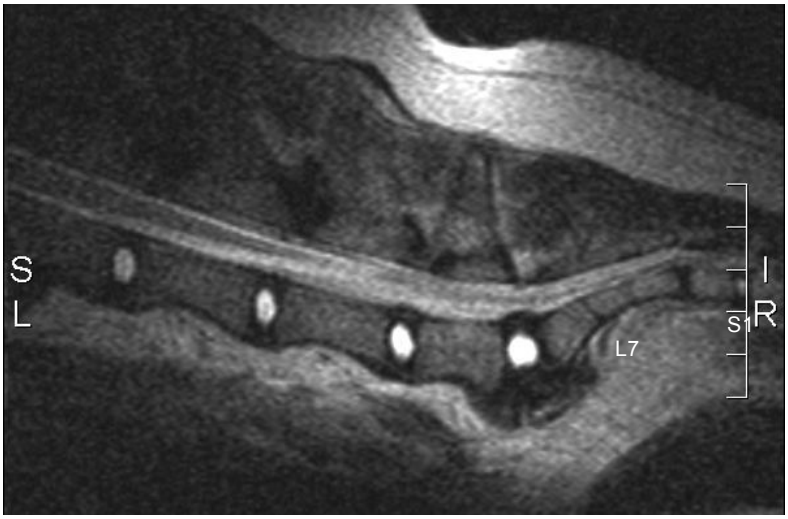

Figure 5: A MR image of the lumbosacral spine of a Boxer with an IVD herniation between L7 and S1 with a Pfirrmann score of 1.

Histological scoring

The histological material was graded according to an adjusted version of the grading system first designed by Boos et al. (2002). The classification system by Boos et al. was developed for age-related histological alterations in human intervertebral discs [4]. When using this system in surgically acquired canine material several parameters needed to be scored differently. The scoring system was adjusted by Dr. G.C.M. Grinwis in concordance with the author (table 4). After some clarifications about IVD histology, the author practiced before beginning the actual scoring. Practice was done on a total of 74 slides, thus on slides which were included (n=44) and excluded (n=30) from the actual research. The pathologist reports were available for each slide and the pathologists main conclusions were checked with the authors observations for accordance until the author was sure a learning curve was no longer present. After this, all histological slides were randomly scored twice with an interval of three days to make sure that there was a no memory bias. Scoring was done at a maximum of two hours at a time to diminish concentration related faults. Slides were first looked at with the 4x lens to get an overview, cells were identified using the 40x lens, whilst the actual categories were scored using the 10x lens. In the category clefts and tears the criteria 1-3 in the annulus could not be judged since the structure of AF tissue obtained during surgery is lost. The 4th criterion was included in the scoring system because in several slides severe tissue defects were noticed in the AF. A value of four points was given to this criterion since the consideration was that such severe cases of AF degeneration should be weighted heavily.

Table 4: The adjusted histological grading scheme for use in surgically acquired canine IVD material.

Annulus Fibrosus: Mean Morphology	
0	Well organized collagen lamellae (75%> distinguishable)
1	Mild disorganization; most lamellar layers still distinguishable (50-75%)
2	Moderate disorganization; 25-75% of the lamellar layers distinguishable
3	No or few (< 25%) distinguishable collagen lamellae
Annulus Fibrosus: Mean Chondroid Metaplasia	
0	No chondrocyte morphology, just spindle shaped fibroblasts.
1	Mild chondroid metaplasia
2	Moderate chondroid metaplasia

3	Marked chondroid metaplasia / scar/tissue defects
Annulus Fibrosus: Tear and Cleft Formation	
0	Absent
1	Rarely present
2	Present in intermediate amounts
3	Abundantly present
4	Scar/tissue defects
Nucleus Pulposus: Cells Chondrocyte Proliferation	
0	No proliferation
1	Increased chondrocyte like cell density
2	Connection of two chondrocytes
3	Small size clones (i.e. several chondrocytes group together, i.e. 2-7 cells)
4	Moderate size clones (i.e. > 8 cells)
5	Huge clones (i.e. 15 cells)
6	Scar/tissue defects
Nucleus Pulposus: Presence of Notochordal Cells	
0	Abundantly present (> 50%)
1	Rarely present (1-50%)
2	Absent
Nucleus Pulposus: Matrix Staining Alcian blue / Picrosirius red	
0	Blue stain dominates
1	Mixture of blue and red staining
2	Red stain dominates

Results

Descriptive statistics
A total of 35 CD and 39 NCD dogs were included. Almost twice as much type 1 HNP were found in comparison to type 2 HNP (table 5)

The CD patients were of the following breeds*: American Cocker spaniel (1x), Beagle (2x), Dachshund (8x), dwarf dachshund (5x), French Bulldog (6x), Jack Russell Terrier (7x), Sealyham terrier (2x), Shih-Tzu (3x), and the Yorkshire terrier (1x).*

And the NCD patients were of these breeds: *American Bulldog (1x), Belgian Shepherd Dog (5x), Bolognese (1x), Bordeaux Dog (1x), Border collie (1x), Boxer (1x), Crossbreed (5x), Dalmatian dog (2x), Nova Scotia Duck Tolling Retriever (1x), Eng. Cocker Spaniel (3x), German Shepherd dog (3x), Golden retriever (1x), Labrador retriever (5x), Maltese (1x), Rhodesian Ridgeback (1x), Rottweiler (4x), Staffordshire bullterrier (1x), and White Shepherd dog (2x).*

Table 5: Number of patients, mean age with standard deviation and standard error of the mean for the tested groups.

Age: CD and NCD dogs

Breed	N	Mean Age	Std. Deviation	Std. Error Mean
CD	35	6.56	3.04	.51
NCD	39	6.58	2.11	.34

Age: Herniation types

Herniation	N	Mean Age	Std, Deviation	Std. Error Mean
Type 1	49	6.72	2.81	.40
Type 2	25	6.29	2.06	.41

When comparing the mean ages of CD and NCD dogs, as well as mean ages between patients with type 1 and type 2 herniations no significant differences were found between the groups (two independent samples T test) as can be seen in table 6.

Table 6: A two independent samples T-test was performed on the mean ages of chondrodystrophic dogs vs. non-chondrodystrophic dogs and on the mean ages of patients with type 1 vs. type 2 herniations. No significant difference between means was found in both tests with P<0.05. This test was ran on the data from table 5.

N=74 ***T-test for Equality of Means***

Breed groups: CD vs. NCD				
Sig, (2-tailed)	Mean Difference	Std, Error Difference	95% Confidence Interval of the Difference	
			Lower	Upper
.99	-.011	.60	-1.21	1.19
Herniation types: type 1 vs. type 2				
Sig, (2-tailed)	Mean Difference	Std, Error Difference	95% Confidence Interval of the Difference	
			Lower	Upper
.49	.43	.63	-.83	1.69

The proportion of type 1 and type 2 herniations in CD and NCD dogs was determined in 74 patients by means of calculating an odd's ratio (table 7) and relative risks.
Relative risks calculations had the following outcomes:
In this group of dogs with clinical IVDD: CD dogs were (32/35)/(17/39)= 2,1 times more likely to have a type 1 herniation than NCD dogs. While NCD dogs were (22/39)/(3/35)= 6,6 times more likely to develop a type 2 herniation than CD dogs. In CD dogs type 1 herniations were seen (Odds) 32/3=10.7 times more often than type 2 herniations. For NCD dogs type 1 herniations were seen (Odds) 22/17= 1.3 times less often than type 2 herniations.
An odd's ratio of (32/3)/(17/22)=13.80 (95% confidence interval 3,61 - 52,83) was calculated for CD dogs having a type 1 herniation, when compared to NCD dogs having a type 1 herniation.

Table 7: The 2x2 table from which the odd's ratio, reverse odd's ratio and relative risks were calculated. Percentages of the total amount of patients are depicted between brackets.

Breed groups vs. herniation types

		Herniation Type		
		Type 1	Type 2	Total
Breed Group	CD	32 (43%)	3 (4%)	35 (47%)
	NCD	17 (23%)	22 (30%)	39 (53%)
	Total	49 (66%)	25 (34%)	74 (100%)

The localizations of IVD herniation for all 74 patients are depicted in figure 6. In this figure several trends can be seen. Type 1 herniations are located more often in the cervical and thoracolumbar area than type 2 herniations. Type 1 herniations are uncommon in the lumbosacral area while type 2 herniations are common in that area. Since CD dogs are most often affected by type 1 herniation and NCD dogs by type 2 herniation, these trends can also be observed for both breed groups. In order to quantify these observed differences, relative risks were determined for the occurrence of type 1 and type 2 herniations in each location and for the occurrence of IVD herniations in the different spinal areas in the two breed groups (CD and NCD).

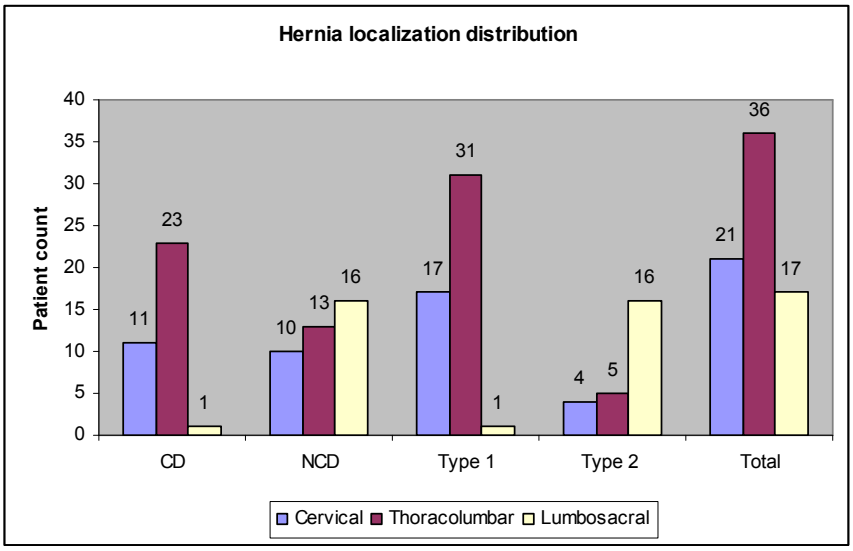

Figure 6: Herniation localizations for all 74 patients.

Relative risks were calculated using table 8.
The probability of a cervical herniation for type 1, when compared to the probability of a cervical herniation for type 2 was (17/49)/(4/25)= 2,2 times larger for type 1.
The probability of a thoracolumbar herniation for type 1, when compared to the probability of a thoracolumbar herniation for type 2 was (31/49)/(5/25)= 3.2 times larger for type 1.
The probability of a lumbosacral herniation for type 1, when compared to the probability of a type 2 herniation was (16/25)/(1/49)= 31.4 times smaller for type 1.

The chance of an IVD herniation in the cervical area for a CD dog, when compared to the chance of an IVD herniation in the cervical area for a NCD dog was (11/35)/(10/39)= 1,2 times larger for a CD dog.
The chance of an IVD herniation in the thoracolumbar area for a CD dog, when compared to the chance of a herniation in the thoracolumbar area for a NCD dog was (23/35)/(13/39)= 2.0 times larger for CD dog.
The chance of an IVD herniation in the lumbosacral area for a CD dog, when compared to the chance of a herniation in the lumbosacral area for a NCD dog was (16/39)/(1/35)= 14.4 times smaller for a CD dog.

Table 8: The herniation localizations for all 74 patients, distributed over the different breed groups and herniation types.

Breed Group	Cervical	Thoracolumbar	Lumbosacral	Total
CD	11	23	1	35
NCD	10	13	16	39
Total	21	36	17	74
Herniation Type	Cervical	Thoracolumbar	Lumbosacral	Total
Type 1	17	31	1	49
Type 2	4	5	16	25
Total	21	36	17	74

Histology

The total scores ranged from 3 till 18, with an average of 10.75. An overview of the mean total scores between histological scoring attempt one and two can be seen in figure 7. Several pictures (figure 7-22) were made to illustrate the different histological scores from the scoring system. They are examples of a certain score in a particular overview although sometimes not representative for the rest of that slide. In the captions beneath the pictures AF means annulus fibrosus and NP means nucleus pulposus. Pictures 7 to 19 were stained with H&E, and pictures 20 to 22 were with Picrosirius red / Alcian blue. A noteworthy finding during the histological scoring was that in NP tissues no fibrocytes were seen by the author.

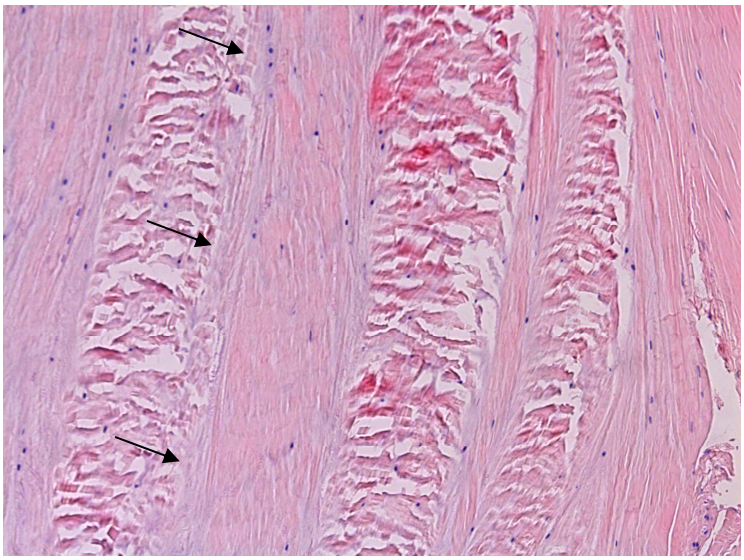

Figure 7: AF morphology, score 0, well organized collagen lamellae (75%> distinguishable). Arrow pointing at a lamellar layer.

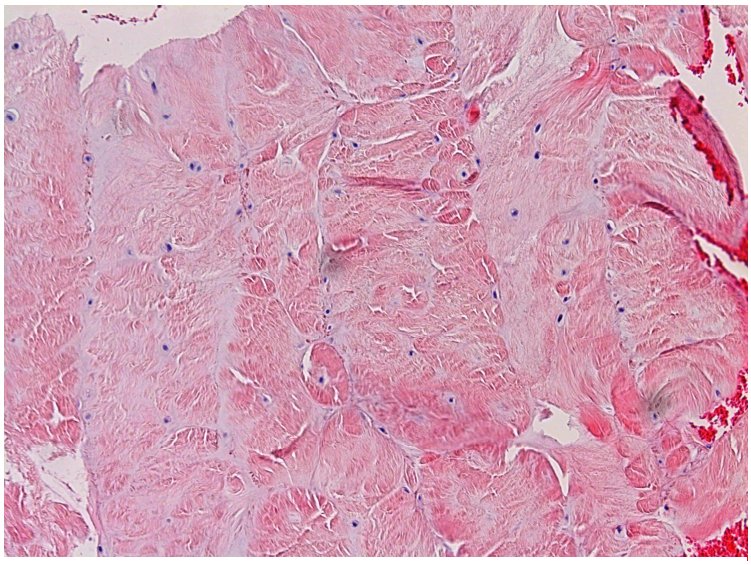

Figure 8: AF morphology, score 1, mild disorganization; most lamellar layers still distinguishable (50-75%).

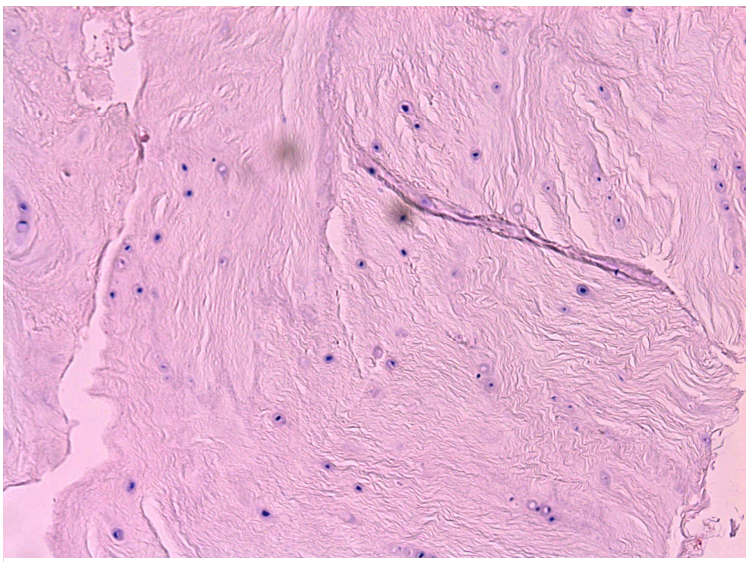

Figure 9: AF morphology, score 2, moderate disorganization; 25-75% of the lamellar layers distinguishable.

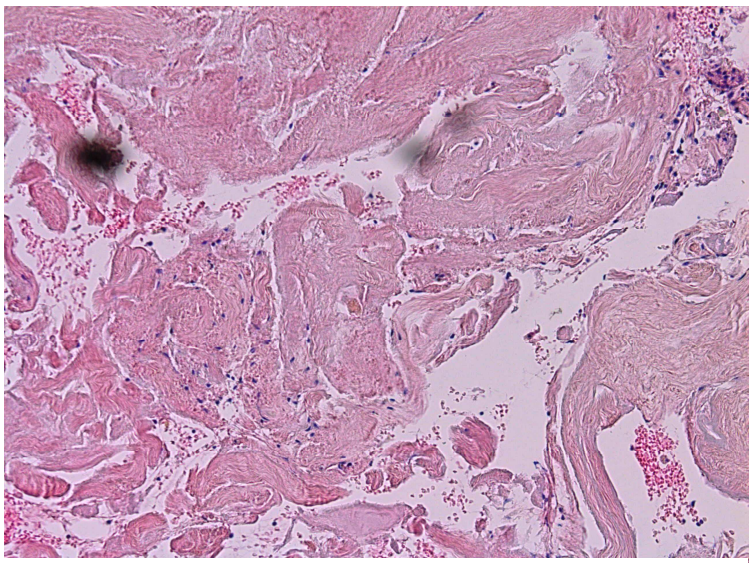

Figure 10: AF morphology, score 3, no or few (< 25%) distinguishable collagen lamellae.

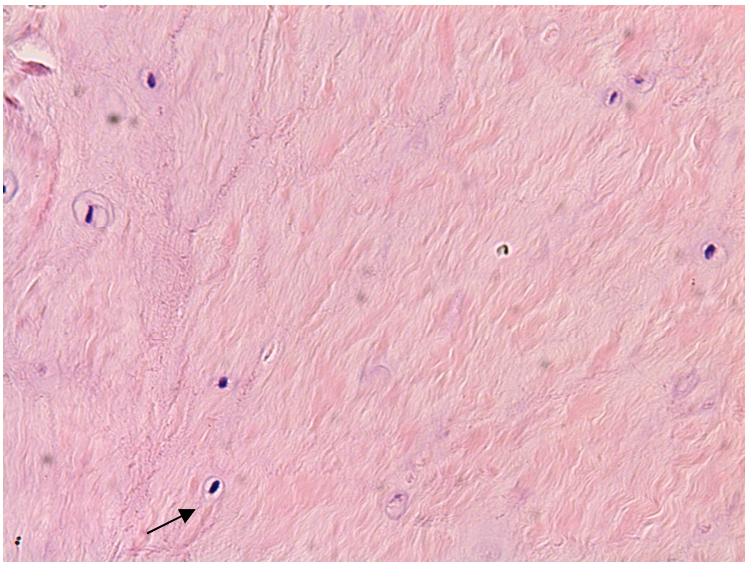

Figure 11: AF chondroid metaplasia, score 1, mild chondroid metaplasia. Arrow pointing at chondrocytes.

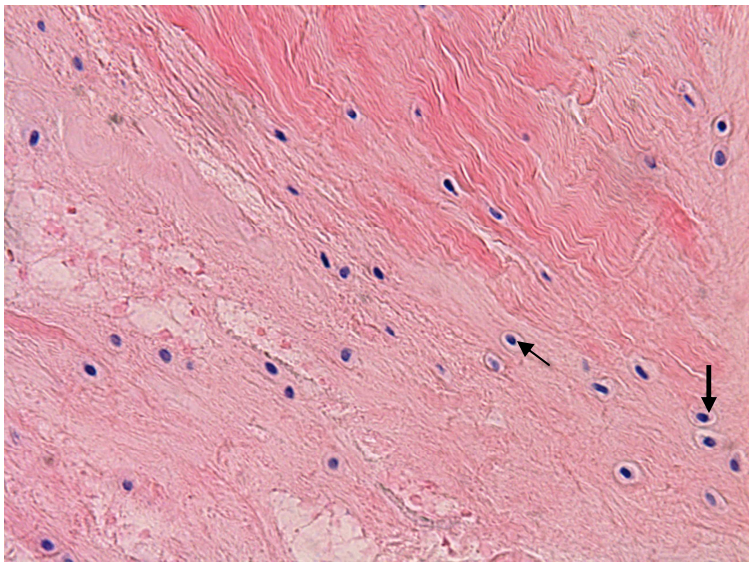

Figure 12: AF chondroid metaplasia, score 2, Moderate chondroid metaplasia. Arrows pointing at chondrocytes.

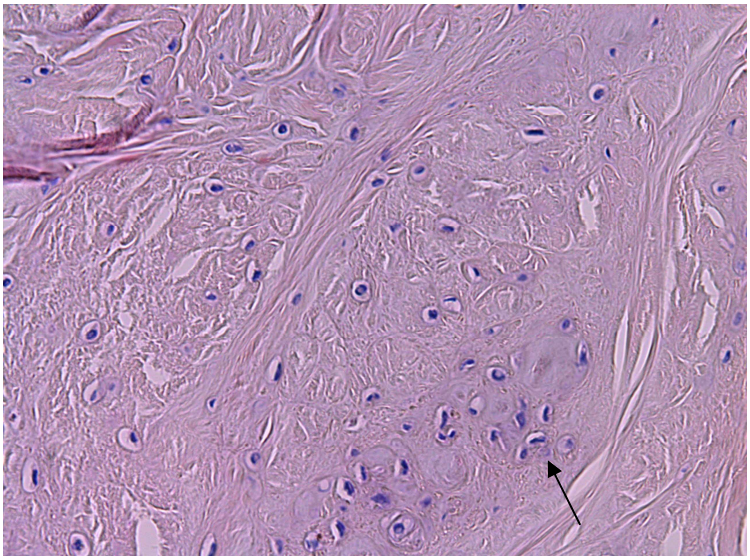

Figure 13: AF chondroid metaplasia, score 3, marked chondroid metaplasia / scar/tissue defects. Arrow pointing at a large cluster of chondrocytes.

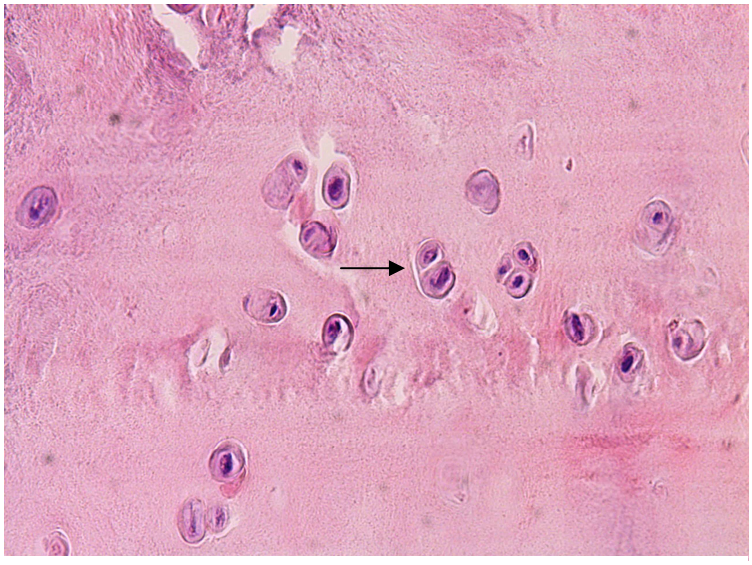

Figure 14: NP Chondrocyte proliferation, score 2, connection of two chondrocytes. Arrow pointing at two connected chondrocytes.

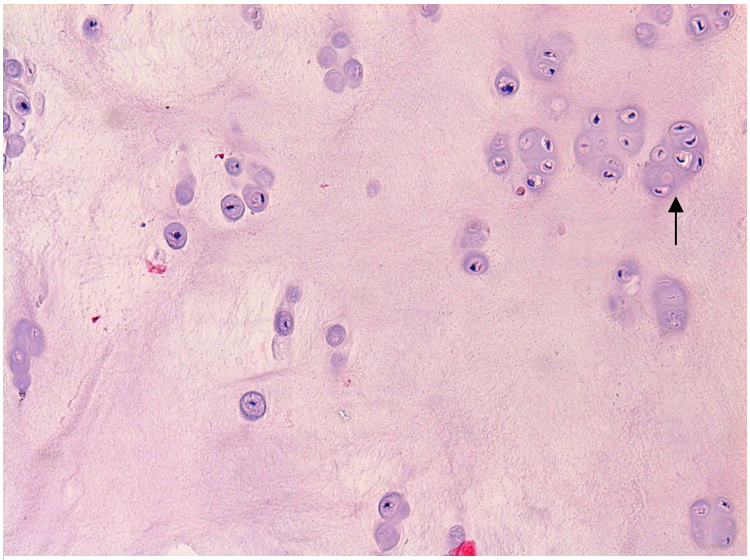

Figure 15: NP chondrocyte proliferation, score 3, small size clones (i.e. several chondrocytes group together, i.e. 2-7 cells). The arrow points at a small size cone of 7 chondrocytes.

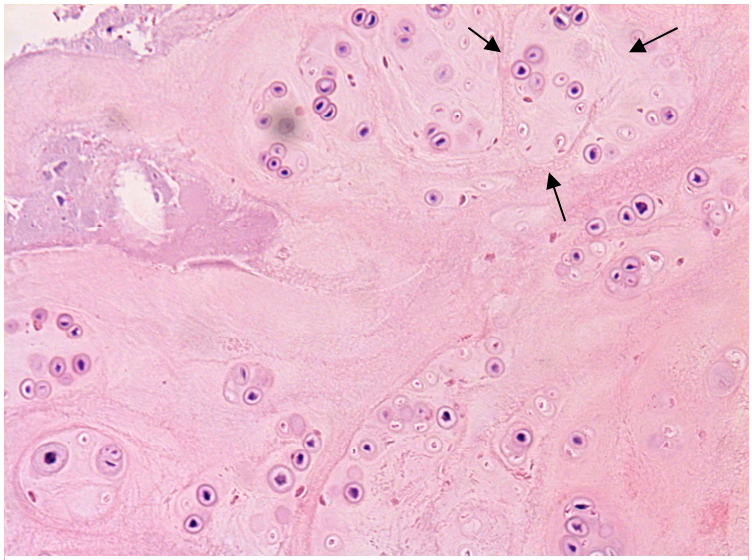

Figure 16: NP chondrocyte proliferation, score 4, moderate size clones (i.e. > 8 cells). The arrows point at a moderate size clone of about 12 chondrocytes. Not the different lacunae.

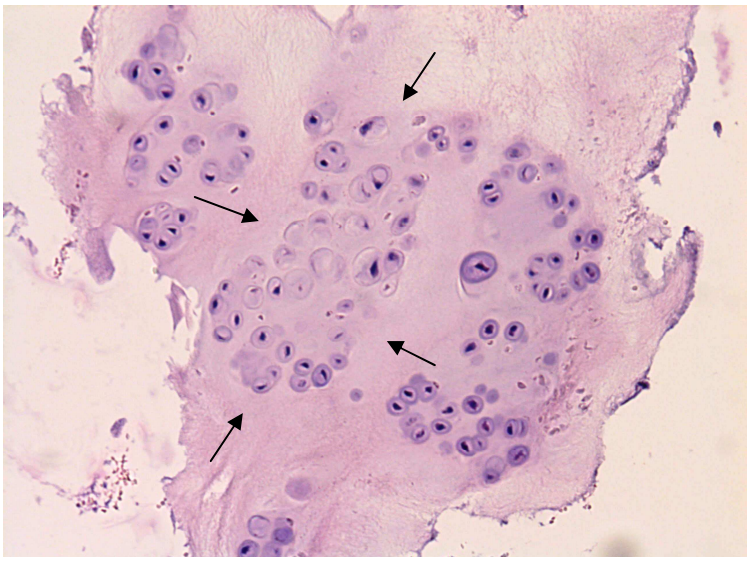

Figure 17: NP chondrocyte proliferation, score 5, Huge clones (i.e. 15 cells). Arrows pointing at a huge clone of over 15 chondrocytes in one lacuna.

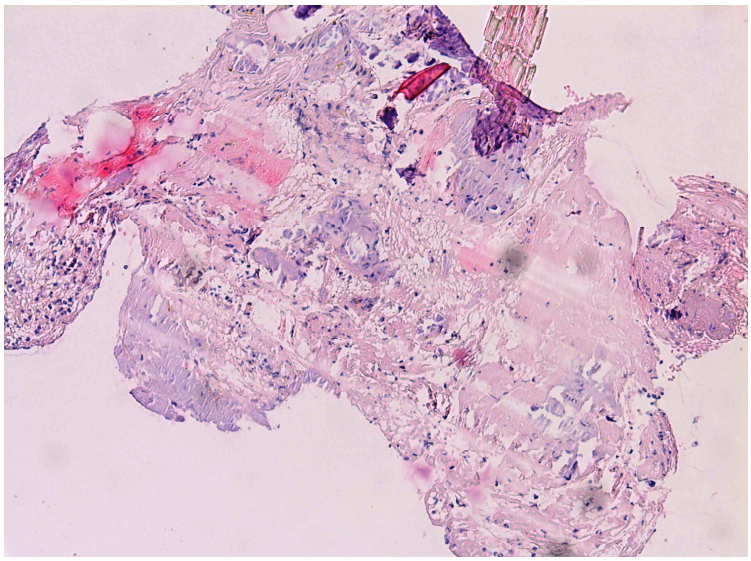

Figure 18: NP chondrocyte proliferation, score 6, Scar/tissue defects. The nucleus tissue is barely recognizable.

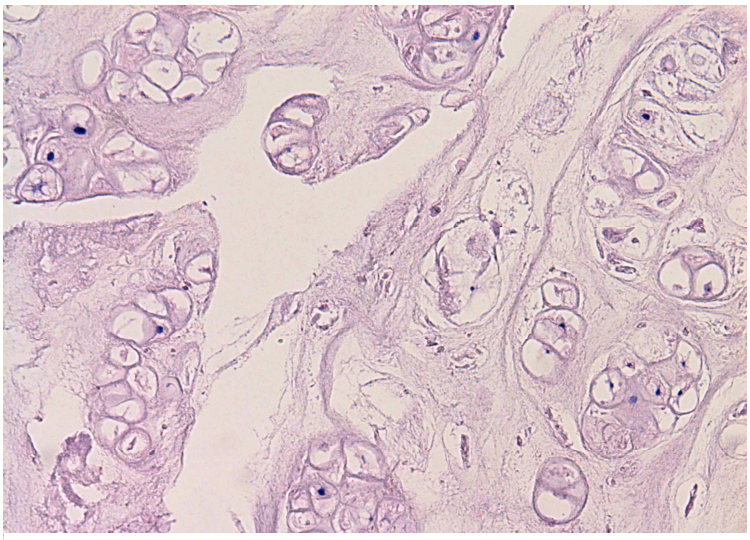

Figure 19: NP notochordal cells, score 0, Abundantly present (> 50%).

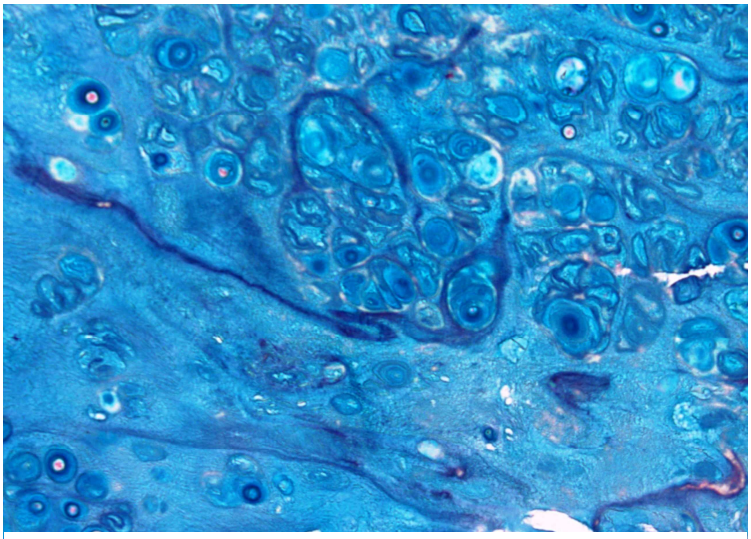

Figure 20: NP matrix staining, score 0, Blue stain dominates.

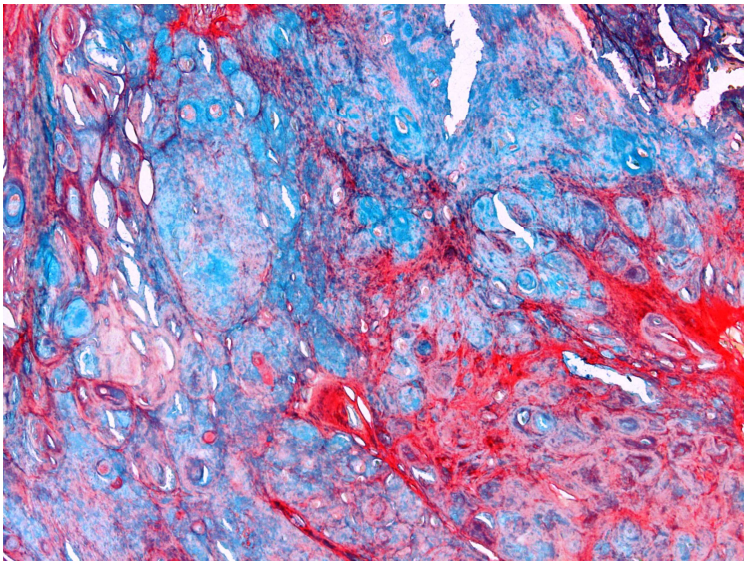

Figure 21: NP Matrix staining, score 1, mixture of blue and red.

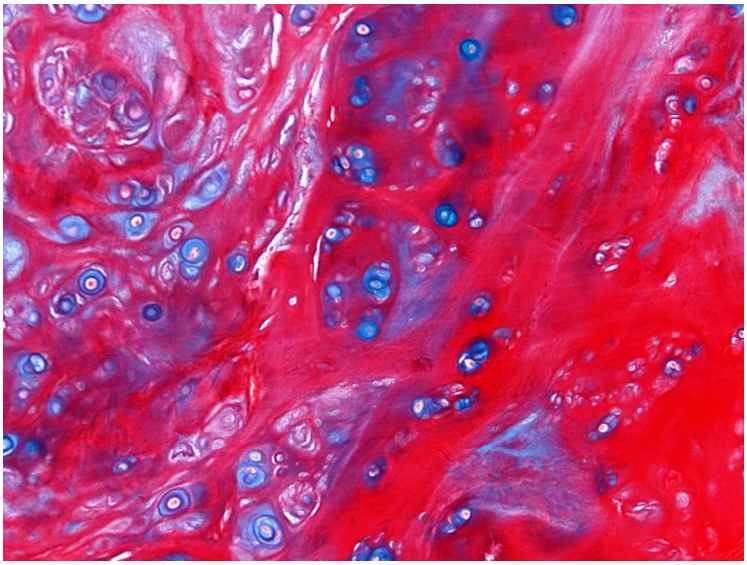

Figure 22: NP Matrix staining, score 2, red stain dominates.

Correlation analysis

Neurological scores were determined for 73 patients. In 73 patients MR images were scored also, 1 patient was excluded from the MRI scoring because of interpretation difficulties caused by birth anomalies. Surgically acquired herniated material consisted of AF and NP, NP only or AF only (table 1). In 44 patients AF and NP were present and these materials were scored histologically. The distribution of the total histological scores, the Pfirrmann scores from scoring attempt one and the neurological scores can be seen in figures 23-25. Non of the scores were normally distributed.

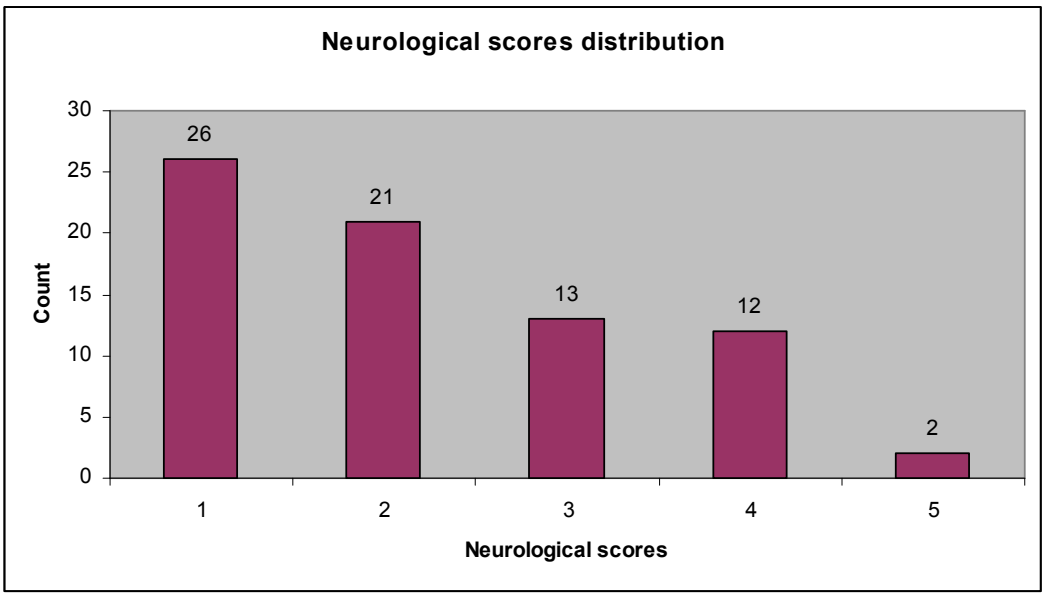

Figure 23: An overview of the number of patients in each clinical neurological scores.

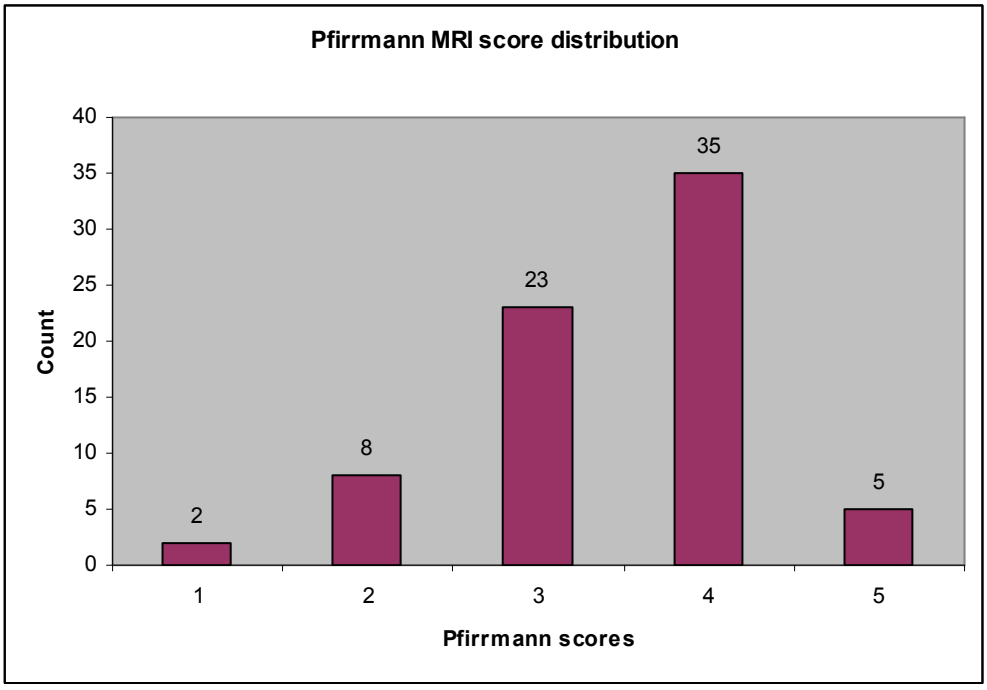

Figure 24: An overview of the Pfirrmann scores from the first MRI scoring attempt.

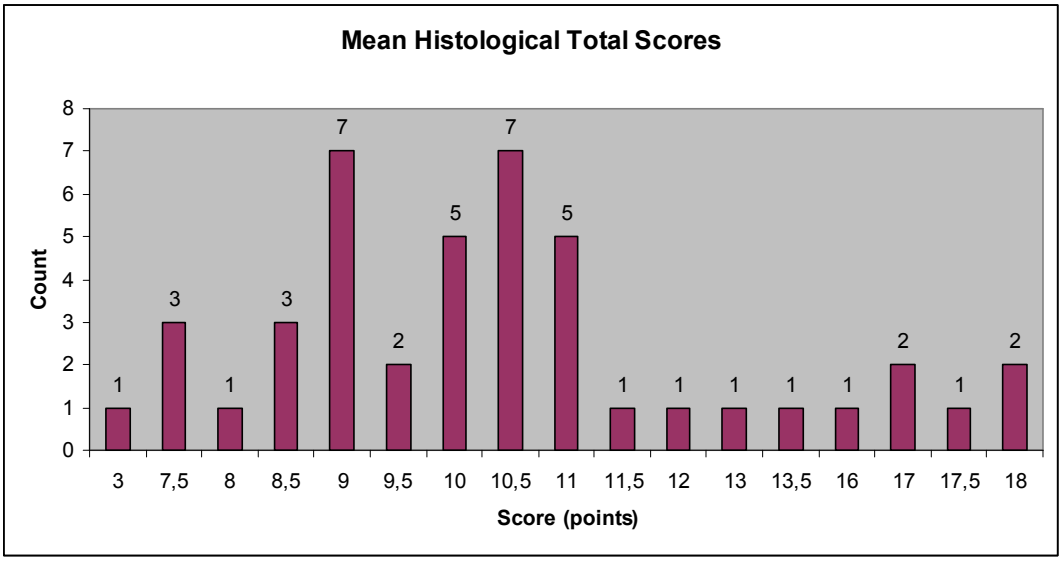

Figure 25: The mean total scores of histological scoring attempt one and two is depicted in this distribution chart. The scale of the horizontal axis is not continues.

All histological slides were scored twice as well as the MR images in order to determine intra-observer reliability. Cohen's weighted kappa was determined for the Pfirrmann grading system and for the histology grading system for each separate category [9]. Intra-observer agreement was excellent for the Pfirrmann grading (κ 0.81-1.00) (table 9) and moderate (κ 0.41-0.60) to substantial (κ 0.61-0.80) for the histological categories (table 10).

Table 9: Cohen's weighted kappa for intra-observer agreement in Pfirrmann scoring. Kappa's 95% confidence intervals is between brackets.

Pfirrmann scoring: intra-observer Kappa *N=73*

Kappa	Standard error	Agreement	1 Grade Disagreement
0,88 (0.64-1.12)	0,12	82%	18%

Table 10: Cohen's weighted kappa for intra-observer agreement in the different histological grading categories. Kappa's 95% confidence intervals between brackets.

Histological grading: intra-observer kappa

N=44	Nr. of categories	Kappa & 95% conf. int.	St. Error	Agreement (%)	1 Grade Disagreement (%)	2 Grades Disagreement (%)
AF: Morphology	4	0,67 (0.38 - 0.96)	0,15	55	43	2
AF: Ch. Metaplasia	4	0,53 (0.24 - 0.82)	0,15	61	32	7
AF: Tear and Cleft Form.	2	0,91 (0.62 - 1.20)	0,15	98	2	0
NP: Chondrocyte Proliferation	7	0,79 (0.52 - 1.06)	0,14	52	45	2
NP: Notochordal Cells	3	0,69 (0.42 - 0.96)	0,14	91	9	0
NP: Matrix Staining	3	0,78 (0.49 - 1.07)	0,15	80	20	0

The total histological scores comprised too many possibilities to calculate a reliable kappa. Because of this, the intra-observer reliability between the two sets of total scores was determined by using correlation analysis. A correlation of 0,68 was found between the first and second histological scoring attempt (Histology 01 and Histology 02) as can be seen in table 11.

Table 11: The correlation was determined between Histology 01 and 02. Correlation is significant at the 0,01 level (2-Tailed).

Correlation between Histological scoring 01 and 02

N=44		Histology 02
Histology 01	Correlation Coefficient	0,68
	Significance (P-value)	<0,001

Correlations were determined between neurological scores, Pfirrmann scores and histological scores.
Since the correlation between histology01 and histology02 was not excellent (0,68) the choice was made not to not only use the average histology score for further analysis but the total scores of both scoring round. For the sake of consistency this was also done for the Pfirrmann scores. In all situations Spearman's rho correlation coefficient was determined since the scores did not have a normal distribution.
For 73 patients the correlations between neurological scores and Pfirrmann score 1 and 2 were determined. None of these correlations were higher than 0,15 and they were non-significant at a P value of 0,05 (table 12).
For 44 patients correlation between neurological scores and histological scores were determined. One significant correlation was found in this comparison – in case of Histology01 – however no significant correlations were found between Histology02 and the mean histology score (table 13) when compared with the neurological scores.
In the comparison made between Pfirrmann scores and histological scores (in 44 patients) significant correlations ranging between 0,30 and 0,51 were found at the 0,01 and 0,05 level, (table 14).

Table 12: Spearman's rho correlation coefficient between neurological and Pfirrmann scores was determined for Pfirrmann scoring attempt 1 and 2 and for the mean Pfirrmann score. The P value is shown between brackets. No significant correlations were found.

Correlation between Neurological Scores and Pfirrmann Scores

N=73		Neurological score (P)
Pfirrmann 01	Correlation Coefficient	0,15 (0,20)
Pfirrmann 02	Correlation Coefficient	0,05 (0,10)
Mean	Correlation Coefficient	0,11 (0,65)

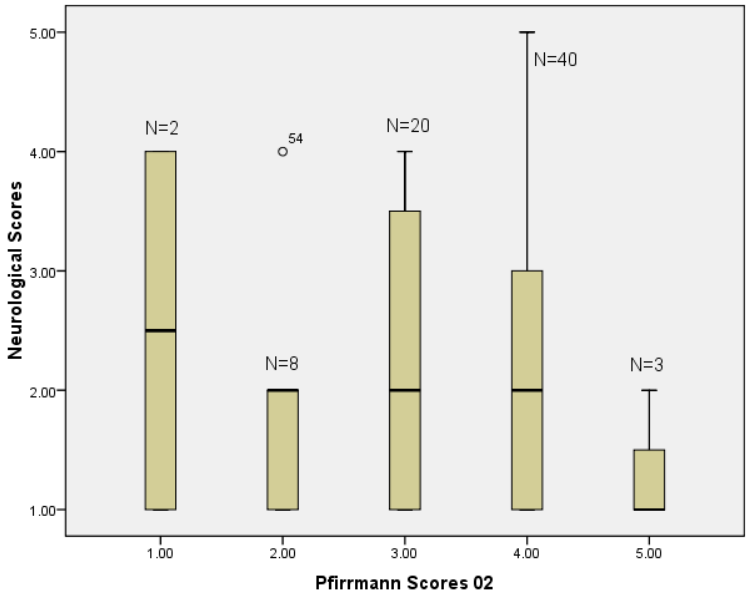

Figure 26: Box and whisker plot of Pfirrmann scoring attempt 2 vs. the neurological scores. No significant correlation was found.

Table 13: Spearman's rho correlation coefficient between neurological scores and histological scoring attempt 1 and 2 and for the mean histological score. The bold correlation is significant a the 0,05 level (2-tailed). P value between brackets.

Correlations between Neurological Scores and Histological Scores

N=44		Neurological Score (P)
Histology 01	Correlation Coefficient	**0,38** (0,01)
Histology 02	Correlation Coefficient	0,15 (0,32)
Mean score	Correlation Coefficient	0,26 (0,09)

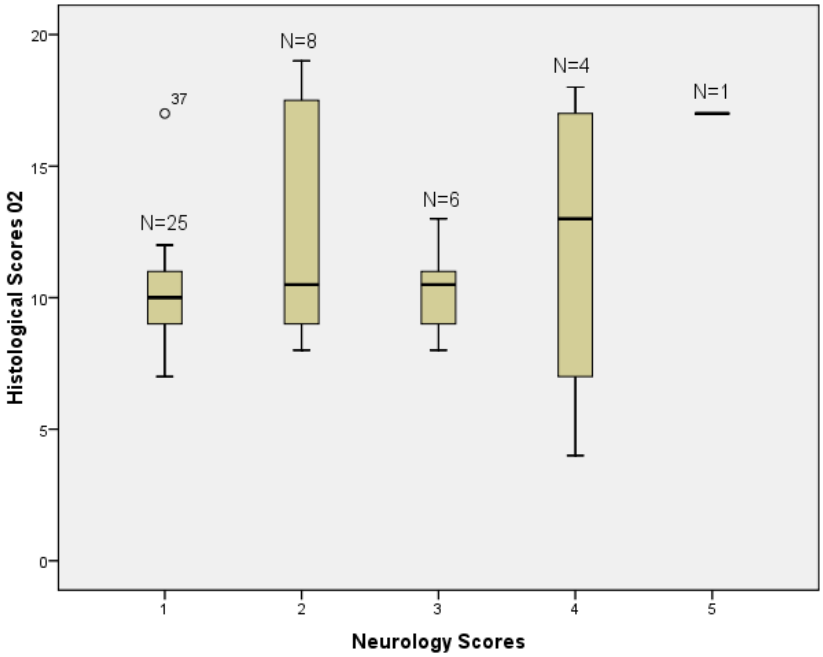

Figure 27: Box and whisker plot for neurological scores vs. histological scoring attempt 2 total scores. No significant correlation was found.

Table 14: Spearman's rho correlation coefficient between histological and Pfirrmann scores. Bold correlations are significant at the 0,01 level (2-tailed) and underlined correlations at the 0,05 level (2-Tailed). Significance is shown between brackets.

Correlations between Pfirrmann Scores and Histological Scores

N=44		Pirrmann 01 (P)	Pfirrmann 02 (P)	Mean score (P)
Histology 01	Correlation Coefficient	**0,51 (<0,001)**	**0,47 (<0,001)**	**0,50 (<0,001)**
Histology 02	Correlation Coefficient	0,29 (0,06)	0,35(0,02)	0,30 (0,05)
Histology mean score	Correlation Coefficient	**0,43 (<0,001)**	**0,45 (<0,001)**	**0,43 (<0,001)**

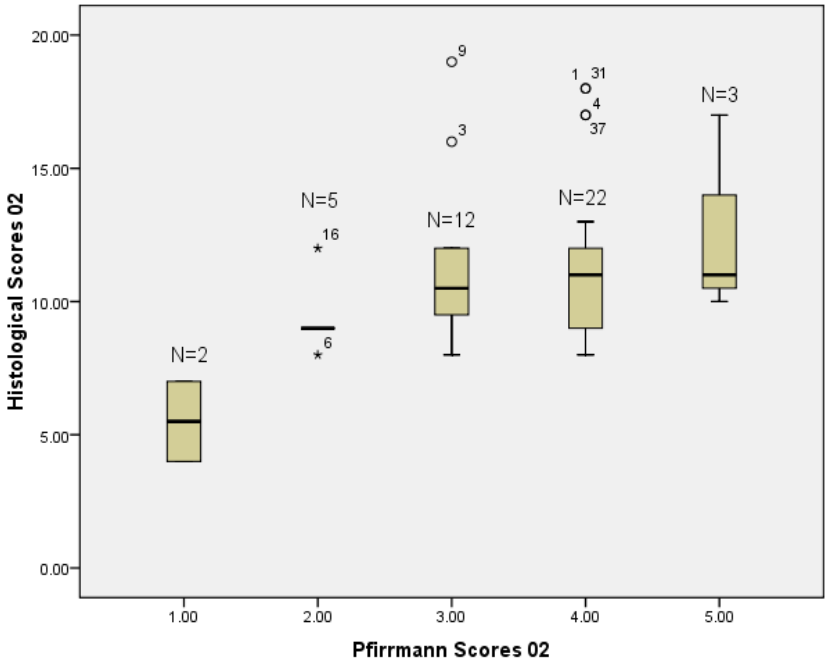

Figure 28: Box and whisker plot of Pfirrmann scoring attempt 2 vs. the total histological score of histological scoring attempt 2. The significant (P<0,05) Spearman's rho correlation coefficient was 0,35.

Discussion

When reading literature on IVDD one can find that type 1 herniations are confined to the CD dog breeds, while type 2 herniations can occur in any type of breed at old age [6,7,16]. The research from Hansen in the 1950's is mostly used as a reference for this finding [6,7,16,18].
Results from the present research project showed, in concordance with what Hansen already reported, that in a group of 74 surgical HNP patients more CD dogs had a type 1 herniation and more NCD dogs had a type 2 herniation (odds' ratio of 13,80).
Interestingly, in our patients, type 2 herniations did not occur at an older mean age than type 1 herniations as reported by others [6,7]. Descriptive statistics performed during this study showed no significant difference between the mean ages of CD dogs vs. NCD dogs and of type 1 vs. type 2 herniations. One must keep in mind however that this is the age at which patients were offered for surgical treatment at the clinic, and not the age of onset of clinical symptoms which is described in the other studies. Patients may have been diagnosed earlier in life with another herniation also. In literature the peak incidence of IVD herniations in CD dogs is reported to be at a much earlier age than in NCD dogs [6]. IVD herniation occurrence in CD dogs was reported to be between 3 and 7 years of age, and in NCD dogs to be between 6 and 8 years of age [7].

The ratio of type 1 and type 2 herniations in 74 HNP patients, subdivided in CD and NCD dogs, *was determined by calculating an odd's ratio* (table 7) and relative risks.
An odd's ratio of 13.80 (3,61 - 52,83) was found for a dog with a type 1 HNP being CD, when compared to a dog with type 1 HNP being NCD. Meaning that dogs with a type 1 HNP have a greater probability of being of a CD breed than of a NCD breed, since the odd's ratio is significantly larger than one.
When looking at the occurrence of the herniation types at specific spinal localizations in the two breed types one can say that herniations in the cervical and thoracolumbar area were seen more often in CD dogs. Herniations in the lumbosacral area were more common in NCD dogs.
In the cervical area most herniations are type 1, i.e. extrusions, this is in concordance with the literature, although type 2 herniations can occur in the cervical area as well

[43,44d]. In literature cervical IVD herniations are also reported to be more common in CD breeds [43]. In our research population we found that dogs with cervical herniations were more often CD than NCD.
Thoracolumbar disc disease is more common in CD dogs, but NCD dogs can be affected as well [44e]. NCD dogs, in particular German Shepherd Dogs suffer most frequently from lumbosacral disc disease [44b]. The higher occurrence of CD dogs with type 1 herniations is possibly caused by a difference in etiopathology of disc degeneration between CD and NCD dogs [6].
What deserves mentioning is that in the lumbosacral area only one herniation was of type 1 and that the same herniation was also the only one in the lumbosacral area in a CD dog (figure 6).
Surgically acquired intervertebral disc material requires a different histological scoring system than when complete intervertebral discs are scored. When using surgically acquired patient material it can be challenging to determine which slides consist of AF and which are of nuclear origin. It is therefore necessary to know in advance which IVD parts can be expected in each slide. For this purpose in the present study already available pathologists reports in which the slide contents were described by a professional veterinary pathologist were used. In the scoring scheme used during histological scoring a note for each slide was added with its contents.
Following an earlier research project with fewer patients [15], this research project was set up, with a larger sample size and a slightly adjusted experimental set up. The choice was made to use a different version of the histological grading scheme than used in the earlier research project [15]. Consequence of this choice turned out to be that of the initial 74 patients only of 44 histological material remained useful for scoring.
When using a scoring system that does not differentiate between the two disc parts this problem may be resolved (such a system was used in the research project report of van Gerwen et al. [15]). A downside of using such a scoring system may be that when looking at MR images the entire disc is scored and not a part of it. When considering such a scoring system one should wonder about the value of a comparison with MR images, especially when only the AF is scored histologically. The AF is not clearly visible on MRI because of its dehydrated state. Moreover it can be interesting to see if NP tissue from type 1 and type 2 IVD herniations are in a different state of degeneration.
The determined correlation between the first and second histological scoring round was 0,68, this was not high enough to commence calculations using only the total score or the total score of the first or second scoring round. A possible explanation for this correlation not being excellent is the way the total scores were build up. For instance a plus-one-point difference in one category combined with a minus-one-point difference in another category leads to a difference of 2 points in the total score. Kappa values of each separate category were moderate to substantial, one can say that this is in concordance with the found correlation of 0.68. Inter-observer reliability was not determined in this research project, but is advisable in future research for further validation of the used histological scoring system.
Between neurological and histological scores a significant correlation was found in only one out of three instances. In the two other comparisons between neurological and histological scores correlations were absent. This discrepancy is attributed to the correlation of 0.68 between the two histological scoring rounds total scores.
Further results showed no significant correlations between neurological and Pfirrmann scores. One explanation for this is that in the Pfirrmann system neuronal tissue compression is not included. Nerve compression is recognized as a cause of pain and neurological deficits. However the pathofysiology of neuronal tissue injuries is multifactorial and thus nerve compression may not be the only cause of symptoms [32]. One can argue that nerve compression should therefore be part of a MRI scoring system for IVD disease. Furthermore, individual differences in pain transduction and perception may play a role as well, together with the co-occurrence of other spinal disorders.
In a study by Ryan et al. a significant correlation between spinal cord compression and neurological symptoms in 33 dogs with cervical type 1 herniations was found [39]. Another study however – by Penning et al. (2006) – showed no association between the degree of spinal cord compression on MR images and the neurological grade in patients

with a thoracolumbar type 1 IVD herniation [32]. The spinal area may be a factor of importance to whether or not a correlation is found.
During this research project I found a significant correlation between Pfirrmann and histological scores, ranging between 0.30 and 0.51. These correlations can be interpreted as weak to moderate, when the Pfirrmann score rises the histological score rises as well and vise versa. This can be seen in the box and whisker plot (figure 28) for the second Pfirrmann scoring round against the second histological scoring round.
With an increase in disc degeneration there is a decrease in proteoglycan synthesis [1]. A direct correlation between proteoglycan content and the brightness of T2W MRI has been found in a study by Pearce (1991), curiously enough no correlation between disc water content and MR signal intensity was found in this study [31]. A possible cause for decreased proteoglycan content is loss of notochordal cells from the NP. When chondrocytes are co-cultured with notochordal cells proteoglycan production increases [1]. In a study that compared proteoglycan concentrations in CD and NCD IVDs a significantly lower proteoglycan content in the NP of CD discs was seen [10]. It is known that notochordal cells disappear early in life in CD dogs [10].
In a study of Seiler et al. (2003) a highly significant association between changes in MRI and histopathological findings in intervertebral discs was found. However they used complete intervertebral discs, different scoring methods, only discs from the lumbar area, and dogs were not known to be suffering from IVDD [42].
Since I had access to the complete thesis by Hansen (1951) [18], this knowledge was kept in mind during the histological scoring. Hansen described two types of histological degeneration: chondroid and fibrinoid. In which the chondroid type occurred more often in CD dogs and the fibrinoid more often in NCD dogs. A noteworthy observation I made while scoring the histological material used in this research project was that I have not noticed any fibrocytes in nucleus pulposus tissue and have therefore not detected the typical fibrinoid degeneration in any of the scored biopsies, neither in CD nor in NCD dogs. The chondroid degeneration type however was observed frequently. It might be that Hansen had noted astray AF cells (fibrocytes) in the NP instead of a completely different degeneration type.
This interesting finding can be the subject of more study, as well as redoing other parts of Hansen's study with the knowledge of today.

Conclusion

The occurrence of two different types of disc herniation at different spinal localizations in CD and NCD breeds was determined in a surgically treated population. Dogs with a type 1 HNP have a greater probability of being of a CD breed than of a NCD breed, since the odd's ratio (13.80) is significantly larger than one. This outcome is comparable with the literature describing IVDD in the canine population at large. Mean ages of CD vs. NCD dogs and of type 1 vs. type 2 herniations showed no significant difference, this outcome is different from what is described in the literature. The outcome in the comparison of the different herniation types in the various spinal area's showed that in the cervical and thoracolumbar areas type 1 herniations occurred more often and in the lumbosacral area type 2 herniations were more common. This was found to be similar in both CD and NCD IVDD patients. In the CD dog group cervical and thoracolumbar herniations were seen more often than in the NCD dog group, while in the included NCD patients lumbosacral herniations were seen more often than in the CD patients.
Correlations, with a maximum correlation coefficient of 0.51, were found between histological and Pfirrmann MRI scores and were rendered significant.
Correlations between neurology and histology and between neurology and Pfirrmann scores were not found. Since there were no correlations between these scores the conclusion can be drawn that Pfirrmann MRI scoring is not useful to evaluate possible clinical signs. And the neurological score of a patient does not predict the amount of histological degeneration seen in that particular disc.
In conclusion, the found moderate correlation (0.51) between Pfirrmann MRI and histological scores cannot be directly used in a clinical setting, but is of value for further

research. Future research can be done to use the amount of MRI and histological degeneration to predict recurrence of IVD herniations in patients. More suggestions for future research are to further optimize the histological scoring system for use in surgically acquired IVD material and to look at the amount of spinal cord compression on MR images instead of or combined with using the Pfirrmann scoring system. The histopathological study of herniated IVD material is still a valuable tool in extending the understanding of the process of disc degeneration.

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