Temporal Characterization and Prognostic Value Determination of Severe Spinal Cord Injuries in Paraplegic Dogs Using \textit{in vivo} Diffusion Tensor Imaging

THESIS

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To Johanna
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</tr>
<tr>
<td>ADC</td>
<td>apparent diffusion coefficient</td>
<td></td>
</tr>
<tr>
<td>AQP-4</td>
<td>aquaporin 4</td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>computer tomography</td>
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<tr>
<td>DPP</td>
<td>deep pain perception</td>
<td></td>
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<tr>
<td>DTI</td>
<td>diffusion tensor imaging</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>fractional anisotropy</td>
<td></td>
</tr>
<tr>
<td>FLAIR</td>
<td>fluid attenuated inversion recovery</td>
<td></td>
</tr>
<tr>
<td>GF</td>
<td>growth factor</td>
<td></td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
<td></td>
</tr>
<tr>
<td>IVD</td>
<td>intervertebral disc</td>
<td></td>
</tr>
<tr>
<td>IVDH</td>
<td>intervertebral disc herniation</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>activated microglia phenotype 1</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>activated microglia phenotype 2</td>
<td></td>
</tr>
<tr>
<td>MFR</td>
<td>motor function recovery</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
<td></td>
</tr>
<tr>
<td>ROC</td>
<td>receiver-operating characteristics</td>
<td></td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
<td></td>
</tr>
<tr>
<td>SCI</td>
<td>spinal cord injury</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the means</td>
<td></td>
</tr>
<tr>
<td>T2W</td>
<td>T2-weighted</td>
<td></td>
</tr>
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1. Chapter 1: Aims of the study

Spinal cord injury (SCI) caused by intervertebral disc herniations (IVDH) or exogenous trauma is a common neurological condition in dogs, which causes impairments of motor, sensory and visceral functions (Fluehmann et al. 2006; Olby et al. 2003). Canine SCI is a well-established large animal translational model for humans affected by traumatic SCI and occurs naturally and spontaneously (Jeffery et al. 2006; Levine et al. 2011). Currently, therapeutic approaches targeted to decrease or detain the secondary wave injury are limited and subject for ongoing research (Raspa et al. 2016). In addition to development of novel treatment strategies, techniques providing prognostic or outcome measurements are a focus of interest.

Diffusion tensor imaging (DTI) is a modality of magnetic resonance imaging (MRI) that enables *in vivo* characterization of axonal tracts by quantifying diffusion of water molecules within the nervous tissue (Vedantam et al. 2014). Directional restriction of diffusion is described by the fractional anisotropy (FA) value, whereas magnitude of diffusion is expressed by apparent diffusion coefficient (ADC) value (Lerner et al. 2014). Since correlation between spinal cord parenchyma destruction and clinical assessment was proven *ex vivo* using histopathology (Henke et al. 2013), DTI could represent a valuable tool for assessment microstructural changes in dogs with severe thoracolumbar SCI.

The aims of the present study are: (1) to prove feasibility of DTI to detect diffusion changes in the acute or chronic injured spinal cord of paraplegic dogs compared to values from control dogs, (2) to describe temporal evolvement of DTI metrics in paraplegic dogs with recovery of motor function after decompressive surgery, (3) to compare values of DTI between acute and
chronic stages of SCI, and (4) to determine the pre-operative prognostic value of DTI in paraplegic dogs with thoracolumbar SCI.
2. Chapter 2: Introduction

2.1. Current knowledge of traumatic spinal cord injury

Spinal cord injury (SCI), a devastating condition affecting the central nervous system, is associated with sensory, motor and visceral function impairment as well as chronic pain (Hagg and Oudega 2006). Further complications include concomitant symptoms such as muscular atrophy, urinary and/or faecal incontinence and urinary tract infections (Cruz et al. 2014; Pavese et al. 2016). Jazayeri and colleagues (2015) reported a worldwide incidence of traumatic SCI in humans ranging from 3.6 to 195.4 affected individuals per million; however, such estimation derives from 41 countries mostly placed in Europe. Therefore, the incidence of SCI could be higher in developing countries. Most common causes of traumatic SCI in humans are vehicular accidents, bullet or other violence related penetrating lesions, sport related traumas and falls, especially in elderly individuals (Dobkin and Havton 2004; Jazayeri et al. 2015).

The first documentation of SCI was described in the Edwin Smith papyrus in 1700 BC. Since that time, understanding of pathophysiologic mechanisms occurring at different time points of the disease is substantially increased; however, therapeutic approaches for patients suffering SCI remain principally palliative (Silva et al. 2014; van Middendorp et al. 2010). Stabilization of the vertebral column in case of fractures or luxations as well as decompression of the spinal cord remain the standard approach for patients affected by traumatic SCI (Fehlings and Perrin 2005). Administration of high corticosteroid dosage has been formerly included in the medical approach; however, a recent placebo-controlled randomized study showed no benefit of administration of methyl prednisolone in paraplegic dogs within 24 hours of onset of paraplegia (Olby et al. 2016).
2.2. Role of the dog as a large animal translational model: bridging the gap between the bench and the bedside

Several animal models have been utilized to expand understanding of SCI, investigate prognostic factors and evaluate novel therapeutic strategies (Hoffman and Dow 2016; Jiang et al. 2016; Oliveri et al. 2014). The rodent model certainly has been the most commonly applied model (Anwar et al. 2016; Silva et al. 2014). In experimental SCI in rodents, a lesion in the spinal cord is produced by contusion, distraction, dislocation, hemisection or transection (Anwar et al. 2016; Wang et al. 2014). A dorsal laminectomy is performed in the thoracic vertebral column for artificial induction of the lesion under general anaesthesia (Jeffery et al. 2006).

Although rodents provide favourable conditions for laboratory research, including easy keeping of animals, population homogeneity and reproducible lesion induction, inherent physiologic differences between species and nature of SCI represent a breakage making direct translation of disease mechanisms and therapeutic approaches between rodents and humans quite difficult (McMahill et al. 2015). Consequently, the necessity of a translational large animal model represents a unique opportunity for the canine model to bridge the gap between laboratory and clinical conditions (Bock et al. 2013). SCI in dogs is often the result of a contusive-compressive lesion caused by a spontaneously occurring intervertebral disc herniation (IVDH) or an exogenous trauma (Fluehmann et al. 2006; Olby et al. 2003). As the dog is naturally and spontaneously affected by SCI, it reliably resembles heterogeneous conditions regarding variations in severity of the lesion, localizations, clinical signs, and histopathologic changes also present in the human counterpart (Jeffery et al. 2006; Levine et al. 2011). Furthermore, the fact that most dogs with SCI are privately owned makes this
species ideal for new therapy implementations and long-term follow up studies (Hoffman and Dow 2016).

2.3. Canine intervertebral disc degeneration and herniation

The healthy intervertebral disc (IVD) consists of a gel-like nucleus pulposus and a transition zone surrounded by an elastic annulus fibrosus and cartilaginous endplates (Fig. 1A; Bergknut et al. 2013b; Pattappa et al. 2012). The nucleus pulposus is composed of water (70-90%), proteoglycans, and collagen type II and its main function lays in balancing compressive pressures deriving from biomechanical forces exerted to vertebral endplates (Buckwalter 1995; Pattappa et al. 2012). Cellularity of IVD is low and consists of chondrocytes, fibroblasts, and notochondral cells, representing only 1% to 2% of the entire tissue volume; nevertheless, cells are essential for maintenance and proper function (Cappello et al. 2006).

In 1951, Hansen described two different types of IVDH. Hansen type I IVDH is characterized by extrusion of degenerated nucleus pulposus into the vertebral canal through a defect in the dorsal aspect of the annulus fibrosus (Fig. 1B). SCI caused by Hansen type I herniations occurs more frequently in chondrodystrophic breeds including Dachshunds, Pekingese, Welch Corgis, Shi Tzu, Lhasa Apso, and French Bulldogs (Bergknut et al. 2012a; Ito et al. 2005; Olby et al. 2004; Priester 1976). Chondrodystrophic dogs present disproportionally short limbs, which are the product of disturbed endochondral ossification, primarily of long bones (Brisson 2010; Hansen 1952; Smolders et al. 2013). Additionally, early intervertebral disc degeneration has been evidenced in the cervical and/or thoracolumbar segment of the vertebral column of young adult chondrodystrophic dogs (Brisson 2010; Hansen 1952; Olby et al. 2004).
Hansen type II herniation is described as elevations of the *ligamentum longitudinale* or bud shape protrusions without involving a complete rupture of the annulus fibrosus (Fig. 1C). The protrusion is caused by fibrous degeneration of the annulus fibrosus and predominantly occurs in non-chondrodystrophic breeds (Hansen 1951). Mechanical stress applied on the IVD leads to thickening of the dorsal part of the disc and eventually to protrusion of the degenerated annulus fibrosus into the vertebral canal. A dorsal protrusion is facilitated as the ventral aspect of the annulus fibrosus is two to three times thicker than the dorsal aspect (Hansen 1952; Jeffery et al. 2013). Hansen type II IVDH is commonly associated with chronic clinical signs (Jeffery et al. 2013).

**Fig. 1:** *Intervertebral disc herniations (IVDH) described by Hansen (1951).* Schematic transversal representation of a healthy intervertebral disc (A), Hansen type I IVDH (B), and Hansen type II IVDH (C). According to Hansen (1951) and Smolders and colleagues (2013).

Histological differences between chondrodystrophic and non-chondrodystrophic dogs have been evidenced within intervertebral discs even in new-borns and IVD degeneration occurs faster in chondrodystrophic than in non-chondrodystrophic dogs (Hansen 1951). Early degeneration of the nucleus pulposus has been observed in dogs at 3 months of age and starts with a premature and accelerated ageing of notochordal cells and chondroid metaplasia at the
periphery and continuing to the whole extension of the nucleus pulposus (Bergknut et al. 2013a; Bergknut et al. 2012b; Cappello et al. 2006). Notochordal cells synthesize proteoglycans associated with the maintenance of extracellular matrix (Pattappa et al. 2012). A reduction of notochondral cells within the nucleus pulposus causes therefore dehydration, decrease of elasticity and finally dystrophic calcification of the whole nucleus pulposus (Jeffery et al. 2013). Furthermore, chondroid metaplasia can be observed in the annulus fibrosus in combination with annular lamellae separation and/or partial rupture (Bergknut et al. 2013a). In chondrodystrophic dogs, annulus fibrosus degeneration is commonly confined to the dorsal and/or dorsolateral aspects with no further related findings in the lateral or ventral aspects (Hansen 1952).

Degeneration of IVD leads to a redistribution of intradiscal pressure points, which combined to additional mechanical stress to a locally debilitated annulus fibrosus may produce sudden extrusion of degenerated nucleus pulposus into the vertebral canal, causing a contusive-compressive lesion (Griffiths 1972; Jeffery et al. 2013).

2.3.1. Clinical classification for dogs affected by thoracolumbar SCI

Canine SCI involves a large variety of neurological deficits ranging from paravertebral hyperaesthesia to paraplegia (Olby et al. 2004). Several clinical scores and subsequent modifications have been established to assess severity of initial clinical signs and locomotor function recovery. With such scores, the clinical status of dogs after SCI is evaluated, reported and compared (Olby et al. 2004; Penning et al. 2006; Sharp and Wheeler 2005; Song et al. 2016). Clinical classification according to Sharp and Wheeler (2005) was consistently applied in this study and is described in Table 1.
Table 1. Clinical score for dogs affected by thoracolumbar SCI. According to Sharp and Wheeler (2005).

<table>
<thead>
<tr>
<th>Grading</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No neurological deficits</td>
</tr>
<tr>
<td>1</td>
<td>Paravertebral hyperaesthesia</td>
</tr>
<tr>
<td>2</td>
<td>Ambulatory paraparesis</td>
</tr>
<tr>
<td>3</td>
<td>Non-ambulatory paraparesis</td>
</tr>
<tr>
<td>4</td>
<td>Paraplegia with presence of deep pain perception</td>
</tr>
<tr>
<td>5</td>
<td>Paraplegia without deep pain perception</td>
</tr>
</tbody>
</table>

2.4. Pathophysiology of SCI

An acute and direct injury to the spinal cord causes immediate death of neurons, astrocytes, oligodendrocytes and endothelial cells within white and grey matter (Hagg and Oudega 2006; Mietto et al. 2015). Immediate parenchymal disruption produced by direct mechanical damage is defined as primary injury (Kwon et al. 2004). Immediately after the primary injury occurs, damage of endothelial cells leads to haemorrhages and swelling of the spinal cord, deriving in failure of auto-regulatory blood flow mechanisms and ischemia (McDonald and Sadowsky 2002). Moreover, a cascade of cellular responses takes place and causes spreading of inflammatory and vascular reactions and consequently expansion of the lesion (Kwon et al. 2004). This spectrum of responses is defined as “secondary injury” and occurs seconds to months after primary injury having direct impact on axonal de- and regeneration as well as adaptive or maladaptive plasticity (Gwak and Hulsebosch 2011; Hagg and Oudega 2006). Key findings of pathophysiological changes occurring during SCI will be briefly mentioned below and are illustrated in figure 2.
Fig. 2: Temporal distribution of key events after spinal cord injury (SCI). Tissue alterations and processes occurring within the spinal cord, primary mechanical or secondary wave injury at acute, subacute and chronic phases. According to Bock et al. (2013), Hu et al. (2010), Mietto et al. (2015), Rowland et al. (2008), and Smith and Jeffery (2006).

2.4.1. Vascular responses and oedema

Disruption of blood vessels during mechanical injury produces foci of petechial haemorrhage, vasoconstriction and intravascular thrombosis and consequently hypoperfusion, loss of autoregulation of blood flow, and ischaemia (Tator and Fehlings 1991). Impaired microcirculation expands from the epicentre of SCI to adjacent segments causing hypoxia and oedema (Hagg and Oudega 2006; Tator and Fehlings 1991). Furthermore, impairment of the local circulatory autoregulation system makes the spinal cord more sensitive and vulnerable to changes in systemic blood pressure, suggesting that systemic vascular effects after traumatic SCI, such as hypotension and bradycardia, could perpetuate hypoxia (Kobrine et al. 1975; Kwon et al. 2004). Sudden reperfusion occurring after a period of spinal cord ischemia may paradoxically exacerbate the inflammatory response and reactive oxygen species liberation (Anwar et al. 2016).
Oedema is defined as excess of water accumulation in tissue parenchyma. In the spinal cord, two types of oedema are described: vasogenic and cytotoxic (Saadoun and Papadopoulos 2010). Vasogenic oedema refers to a net flow of fluid escaping through a disrupted blood-spinal cord barrier into the interstitial compartment, whereas cytotoxic oedema is the increase of water content through an intact blood-spinal cord barrier into the intracellular compartment (Saadoun and Papadopoulos 2010). Axonal and microglial swelling is evident at early stages of acute SCI and upregulation of water channels in astrocytic cellular membrane, specifically aquaporin-4 (AQP-4), may play an important role in development of cytotoxic oedema and blockage of action potentials (Rowland et al. 2008; Saadoun et al. 2008; Wang et al. 2009). Mechanisms of oedema elimination from lesion site are still unclear; however, Saadoun and Papadopoulos (2010) suggested that excess of water is eliminated parallel to white matter tracts via intra and extracellular AQP-4-independent routes. Both, haemorrhage and oedema during the acute state of SCI are causative factors for parenchymal swelling and ischemia (Rowland et al. 2008).

### 2.4.2. Inflammation

Inflammatory cell migration and infiltration into the spinal cord after primary injury is stimulated by increased expression of leukocyte adhesion molecules from damaged blood vessels (Mietto et al. 2015). Secondary injury after SCI encompasses cellular components from innate (neutrophils, monocytes, and macrophages), resident CNS (astrocytes and microglia), and adaptive immune responses (B and T lymphocytes), and non-cellular components including prostaglandins, cytokines and complement proteins (Anwar et al. 2016; Kwon et al. 2004). After acute SCI, lesioned parenchyma facilitates activation of resident microglia and release of neutrophil chemoattractant molecules through damage-associated molecular patterns (Kolaczkowska and Kubes 2013). Neutrophils are therefore the first
cellular line to migrate into the lesioned spinal cord attracted by E- and P selectins present in endothelial cells (Williams et al. 2011). Once activated, neutrophils are capable of secreting proteolytic enzymes and proinflammatory cytokines and subsequently produce more endothelial damage and chemoattraction (Mietto et al. 2015).

Microglia cells play an important role in perpetuating tissue damage or enabling regeneration after being activated in two different phenotypes, M1 and M2 (Anwar et al. 2016). Activated microglia can be found within and in the periphery of the epicentre and within axonal bundles that undergo Wallerian degeneration (Hagg and Oudega 2006). M1 phenotype is associated with secretion of pro-inflammatory cytokines such as interleukin (IL) -1β, IL-6, interferon -gamma and tumour necrosis factor - alpha, nitric oxide, oxygen reactive species, and glutamate (Anwar et al. 2016). M2 activated microglia are associated with cell survival and axonal regeneration due to release of anti-inflammatory cytokines, including IL-4 and IL-13, and production of several growth factors (GF) such as nerve GF, ciliary GF and epidermal GF (Anwar et al. 2016). Moreover, monocytes and macrophages migrating from the periphery into the spinal cord become indistinguishable from activated microglia cells and their function is phagocytosis of dead cells and tissue debris and secretion of pro- and anti-inflammatory molecules and neurotrophic factors (Mietto et al. 2015).

2.4.3. Excitotoxicity and oxidative stress

Excitotoxicity is defined as a major nerve cell damage and death caused by increased glutamate levels or excessive stimulation of glutamate receptors (Mehta et al. 2013). An overreaction of glutamate receptors due to elevated glutamate levels leads to water influx, cytoplasmic vacuolization, ionic imbalances and finally cell lysis (Hagg and Oudega 2006; Matyja et al. 2005).
Chapter 2: Introduction

Reactive oxygen species (ROS) and free radicals are predominantly secreted by activated microglia and peripheral leucocytes that migrated into the lesion site after primary injury occurs in the human and canine spinal cord (Anwar et al. 2016; Boekhoff et al. 2012a). Polyunsaturated lipids within the cellular membrane are target of reactive lipid peroxidation by free radicals, triggering disruption of the cellular membrane and formation of aldehyde products (Silva et al. 2014).

2.4.4. Apoptosis

Programmed cell death after SCI involves all populations of cells present in the nervous tissue (Silva et al. 2014). However, oligodendrocytes seem to be more susceptible to undergo apoptosis produced by activation of Fas receptors by activated microglial cells expressing Fas ligand (McDonald and Sadowsky 2002; Rowland et al. 2008). This process may continue for days or weeks after SCI, playing a major role in long axonal tract demyelination and perpetuation of Wallerian degeneration (Beattie et al. 2000).

2.4.5. Glial scar formation

Glial scar and fluid filled cavity formation after SCI is considered a common finding of the chronic state and is principally carried out by reactive and hypertrophied astrocytes (Hu et al. 2010). An explanation for this astrocytic response is yet not completely understood; however, it presumably occurs as an attempt to re-establish a barrier between CNS and the rest of the body (Hagg and Oudega 2006). Diminished capacity of the spinal cord to regenerate injured axonal tracts has been attributed to the presence of glial scars (Ohtake and Li 2015). The three dimensional distribution and extension of the scar represent a mechanical and molecular obstacle for neuronal growth since several inhibitory molecules are produced by oligodendrocytes and meningeal fibroblasts (Fawcett and Asher 1999).
2.5. Histopathology

Canine SCI displays a wide variability of histopathological findings (Levine et al. 2011). Alterations evident in histological evaluations frequently correlate with neurological deficits (Henke et al. 2013; Levine et al. 2011).

Predominant findings present during the acute phase are frequently restricted to the epicentre of the lesion and include neuronal injury, inflammation, vasogenic oedema, cytotoxic oedema expressed as axonal swelling in the ventral and ventrolateral long white matter tracts, demyelination and variable degrees of grey matter necrosis and haemorrhage (Bock et al. 2013; Griffiths 1972; Levine et al. 2011; Smith and Jeffery 2006). Furthermore, ultrastructural evaluation using electronic microscopy revealed increased periaxonal space, accumulation and morphological alterations of intraaxonal organelles even in normal appearing axons, especially mitochondria (Bock et al. 2013; Smith and Jeffery 2006).

In the subacute state of SCI, axonal swelling continues to be an important feature as it shows a tendency to affect more tracts and to spread in both directions, cranially and caudally to lesion epicentre (Bock et al. 2013). Presence of phagocytic microglia/microphages increases, foci of haemorrhage and necrosis tend to diminish and a rudimentary cavity formation can be found at this time point (Bock et al. 2013; Hu et al. 2010). Furthermore, degenerative processes continue to take place and clusters of demyelinated axons combined with fragmented myelin are found (Smith and Jeffery 2006).

After consolidation of glial scars, nearly 4 weeks after initial injury and lasting from weeks to months, the chronic status takes place (Hu et al. 2010). Fluid filled cavitations surrounded by an astroglial scar are a common finding in severe chronic SCI (Levine et al. 2011). Smith and Jeffery (2006) described interconnected cell processes, microglia/macrophages and partially
remyelinated axons within these intramedullary cavitations. Presence of Schwann cell mediated remyelination was reported to occur in more densely packed axons surrounding the lesion (Smith and Jeffery 2006). Wallerian degeneration is also a characteristic feature in both species, humans and dogs, and affects more commonly regions caudal to the epicentre than cranial ones (Bock et al. 2013; Griffiths 1972; Hagg and Oudega 2006; Levine et al. 2011).

2.6. The role of diagnostic imaging during SCI

Diagnostic imaging techniques have an important role in the diagnosis of SCI and permit a better understanding of SCI pathophysiology. Plain radiographs are generally performed in dogs with suspected SCI to assess bone structure integrity and findings related to IVDH, such as reduced intervertebral and articular processes spaces; however, they provide only limited indirect information on status of the spinal cord (Jeffery et al. 2013). Myelography consists of an injection of contrast medium in the subarachnoidal space in order to assess the spinal cord compression (Robertson and Thrall 2011). Formerly, myelography represented a common approach for diagnosis of extradural, intradural-extramедullary or intramedullary lesions; however, with increased availability of less invasive, safer and more sensitive techniques such as computed tomography (CT) and MRI, this approach has been replaced (Jeffery et al. 2013; Newcomb et al. 2012; Olby et al. 2000; Robertson and Thrall 2011). MRI has a higher sensitivity and specificity in comparison to other mentioned techniques (Cooper et al. 2014).

2.6.1. Magnetic resonance imaging

Implementation of MRI for diagnosis of SCI enabled not only the detection of the compression site, but evaluation of spinal parenchymal tissue, nerve roots, intervertebral discs, vertebral venous sinuses and ligaments (Dennis 2011; Jeffery et al. 2013; Kube and
Olby 2008). MRI is considered the gold standard for diagnosis and localization of spinal cord injuries in dogs, especially when no alterations are found in plain radiographs or CT scans (Ito et al. 2005; Kube and Olby 2008).

In IVDH, this technique is particularly valuable for identifying and describing extradural haemorrhage or disc material compressing the spinal cord (Fig. 3A). Moreover, intramedullary signal intensity changes in T2-weighted (T2W) sequences have been associated with different pathological processes depending on the temporal stage of SCI (Hu et al. 2010). In the acute stage of SCI, hyperintense signal in T2W sequences has been associated with inflammation, haemorrhage and oedema (Katzberg et al. 1999). In chronic SCI, T2W hyperintensities represent most commonly fluid content within cavitations, myelomalacia and extended Wallerian degeneration (Fig. 3B; Yamashita et al. 1990).
**Fig. 3:** Sagittal and transversal T2-weighted planes of the thoracolumbar spinal cord in acute and chronic stages of spinal cord injury (SCI). Yellow intermittent lines in the sagittal planes indicate positioning of transversal images. (A) acute SCI caused by intervertebral disc herniation (IVDH; 0 days after onset of paraplegia) in a 5.3 years old female dachshund weighting 5.6 kg. Red arrow points to the extruded disc material present in the vertebral canal. (B) chronic SCI caused by IVDH (5 months after onset of paraplegia) in a 4 years old male dachshund weighting 3.5 kg. Extensive intramedullary hyperintense signals are evident; green arrowhead points to an intramedullary cavitation containing cerebrospinal fluid isointense fluid.

### 2.6.2. Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is a modality of magnetic resonance imaging that allows *in vivo* tissue characterization by quantifying water molecule diffusion (Beaulieu 2002). In the spinal cord, cellular membranes of axonal tracts, intraaxonal microtubules and myelin sheaths represent microstructural barriers and provide a homogeneous environment for water molecule diffusion, which takes place predominantly parallel to axons in a cranio-caudal direction (Beaulieu 2002; Sasiadek et al. 2012). This technique also allows three-dimensional reconstructions of white matter tracts with the so called “fibre tracking” (Lerner et al. 2014). DTI and fibre tracking were initially performed in patients before undergoing brain tumour
extirpation, allowing precise preoperative planning of surgical approach and herewith, intending to preserve integrity of important sensory, motor and cognitive centres (Potgieser et al. 2014). However, as a consequence of improvements in acquisition protocols and increase in availability of high-field magnets in diagnostic centres, DTI of the spinal cord has increasingly become a focus of interest during the last decade (Martin et al. 2016; Vedantam et al. 2014).

2.6.2.1. Principles of DTI

Diffusion is a physical property in which molecules undergo randomly and thermically dependent translational movement or Brownian motion (Beaulieu 2002; Potgieser et al. 2014). Through application of magnetic field gradients, MRI may be sensitized for detection of molecule driven motion in the direction of the field gradient (Jellison et al. 2004). Furthermore, using matrix calculations from individual diffusion measurements, the detection of the magnetic field signal is converted into a three dimensional diffusion model called diffusion tensor (Jellison et al. 2004). Generated diffusion tensors within a single image voxel may be visualized as ellipsoids containing three major axis of direction in space or eigenvectors ($\varepsilon_1$, $\varepsilon_2$, and $\varepsilon_3$; Fig. 4); moreover, diffusion tensors enable estimations of diffusivity in any arbitrary direction (Jellison et al. 2004; Lerner et al. 2014). For each direction indicated by eigenvectors, one diffusivity value, or eigenvalue ($\lambda_1$, $\lambda_2$, and $\lambda_3$) is assigned; therefore, representation for diffusivity and direction of diffusion are applied for each ellipsoid (Lerner et al. 2014).

In pure water or cerebrospinal fluid (CSF), where no defined microstructural boundaries are found, diffusion is random and unrestricted, or isotropic, meaning that eigenvalues will have similar magnitudes (Fig. 4A; Lerner et al. 2014). In contrast, anisotropic diffusion is defined
as diffusion dependence on directionality and it may be restricted by highly organized tissues such as white matter tracts (Fig. 4B; Beaulieu 2002; Vedantam et al. 2014).

Fig. 4: Diffusion tensor ellipsoids. (A) Directionally unrestricted (or equally restricted) diffusion forms a spherical tensor. (B) Directionally restricted diffusion present in white matter tracts derive in an ellipsoid tensor with preferential direction parallel to axonal tracts. According to Jellison et al. (2004) and Lerner et al. (2014).

The most common diffusion metrics reported are fractional anisotropy (FA) and apparent diffusion coefficient (ADC; Vedantam et al. 2014). FA is a scalar value from 0 to 1, which derives from information provided by eigenvectors about direction of maximum diffusion within a voxel, whereas ADC values or mean diffusivity values (MD) are magnitude values expressed in $10^{-3}\text{mm}^2/\text{s}$ and is calculated as the mathematical average of the three principal eigenvalues as shown in the equations below (Facon et al. 2005; Lerner et al. 2014; Vedantam et al. 2014).
\[ FA = \sqrt{\frac{2}{3}} \sqrt{\frac{(\lambda_1 - \lambda)^2 + (\lambda_2 - \lambda)^2 + (\lambda_3 - \lambda)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \]

\[ ADC = (\lambda_1 + \lambda_2 + \lambda_3)/3 \]

When applied to all voxels within a sequence, diffusion tensors are able to display preference of diffusion direction from each individual voxel in colour maps, or FA maps. Voxels having a preferential diffusion in the cranio-caudal axis are displayed blue, in the latero-lateral axis red and in the dorso-ventral axis green (Hobert et al. 2013). Furthermore, several consecutive voxels sharing similar diffusion magnitudes and orientation are identified and interpreted as tracts for fibre tractography technique (Facon et al. 2005).

2.6.2.2. DTI of the spinal cord

Diffusion tensor MRI has been extensively described in animal models, mainly in rodents, with acute lesions in the spinal cord induced via contusion, hemi- or total transection (Li et al. 2015; Wang et al. 2014). Performance of DTI in rodents has allowed a better understanding in lesion temporality after different severities of injury (Li et al. 2015; Wang et al. 2014). Nonetheless, this model faces two main obstacles regarding the technique: firstly, a compressive lesion has not been taken into consideration probably because of the small diameter of the rodent spinal cord; and secondly, many DTI evaluations are reported to be performed ex vivo, which permits a reduction in image artefacts but at the same time widens the gap between laboratory and practical conditions (Jirjis et al. 2013; Kelley et al. 2014).

Feasibility of DTI and several protocols have been formerly tested in dogs without any neurological disorder compromising the spinal cord (Griffin et al. 2013; Hobert et al. 2013).
Pease and Miller (2011) reported DTI and fibre tracking of six dogs affected by different spinal cord pathologies (five in the cervical region, one in the thoracolumbar region) such as acute IVDH, chronic IVDH, extradural neoplasia and ischemic myelopathy.

DTI has increasingly gained popularity among the state-of-the-art MRI modalities to evaluate human spinal cord (Martin et al. 2016). Several spinal cord diseases such as multiple sclerosis, amyotrophic lateral sclerosis, cervical spondylotic myelopathy and chronic SCI have been widely studied using DTI in humans (Li et al. 2016; Martin et al. 2016). However, reports regarding acute state of SCI are rare, evaluating inhomogeneous populations and using different methodologies (Cheran et al. 2011; Facon et al. 2005; Vedantam et al. 2013). Evaluating the acute severely injured spinal cord in clinical conditions may be challenging, as most patients require immediate stabilization of the vertebral column or decompression of the spinal cord.

2.7. Assessment of prognosis for acute SCI

Assessment of prognosis in paraplegic humans and dogs is currently performed using clinical tests and are subjectively interpreted (Cruz et al. 2014; Griffin et al. 2009). Clinical evaluation for presence or absence of DPP is accepted as the gold standard; however, interpretation of this test becomes more difficult in dogs showing a “decreased deep pain nociception” (Aikawa et al. 2012).

The search for objective prognostic tools that could reveal an objective and reliable prognosis for paraplegic dogs after SCI has directed research to several fields. Different biomarkers in CSF including tau protein, myelin basic protein, lactate, and matrix metalloprotease-9 have been evaluated; however, none of them has revealed a specificity or sensitivity high enough to replace the clinical assessment (Levine et al. 2010; Roerig et al. 2013; Witsberger et al. 2012).
Degree of spinal cord compression and presence and length of intramedullary hyperintense signal in T2W sequences have also been explored as possible prognostic indicators (Boekhoff et al. 2012b; Ito et al. 2005; Penning et al. 2006). Degree of compression showed no correlation with patient outcome, whereas length of intramedullary hyperintensities used as an indirect indicator for parenchymal damage did show a correlation with DPP and outcome (Boekhoff et al. 2012b; Ito et al. 2005; Penning et al. 2006).

Henke and colleagues (2013) reported a correlation between absence of DPP and severity of tissue destruction in histopathologic evaluations; however, this finding was not consistent in all cases, making in vivo determination of prognosis even more challenging.
3. Chapter 3: Temporal Evolvement of DTI Metrics in Paraplegic Dogs

A. Wang-Leandro performed acquisition of images, generated and analysed the data, and wrote the manuscript.

M. K. Hobert: acquisition of images, advice concerning methods.

S. Kramer: performed partly surgical approaches.

P. Dziallas: performed acquisition of images.

K. Rohn: performed statistical analysis.

V. M. Stein: was involved in the concept and design of the study and critically revised the manuscript, performed partly surgical approaches.

A. Tipold: was involved in designing of the study, discussing analysis of the data and critically revised the manuscript.

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Temporal Evolvement of Diffusion Tensor Imaging Findings in Paraplegic Dogs with Spinal Cord injury and Motor Function Recovery

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Abstract

Traumatic spinal cord injury (SCI) derives in sensory and motor function impairments and represents substantial social and economic burdens. Diffusion tensor imaging (DTI) allows in vivo microstructural evaluation of the spinal cord. Therefore, we measured values of fractional anisotropy (FA) and apparent diffusion coefficient (ADC) of the spinal cord in 19 paraplegic dogs after acute or subacute SCI caused by intervertebral disc herniation (IVDH) and 6 control dogs. All affected dogs underwent surgical decompression of the spinal cord and regained motor function within 4 weeks thereafter. MRI scans were performed preoperative and 3 months after motor function recovery was observed. DTI metrics were obtained at the lesion epicentre(s) and one vertebral body cranially and caudally. Variance analyses were performed to compare values between evaluated localizations in affected dogs and controls and between time points for each localization independently.

At the preoperative time point, an increase of FA values (p=0.0039) together with a decrease of ADC values (p=0.0003) was found in the epicentres and a decrease of ADC was found perilesional (p=0.0235 cranially and p=0.0100 caudally), compared to control dogs. In follow up examinations, no significant differences could be found between DTI values from dogs that recovered motor function and control dogs. Findings suggest that compressive component during IVDH may play an important role in diffusion dynamics during acute and subacute states in natural occurring canine SCI as a translational model for traumatic SCI in humans. Additionally, FA and ADC could be potential markers for cytotoxic oedema and intra axonal ultrastructural alterations found in canine SCI. DTI could be a useful tool for follow-up studies to examine the spinal cord in vivo during recovery phase and/or after novel therapy implementations.
Chapter 3: Temporal Evolvement of DTI Metrics in Paraplegic Dogs

Key words

MRI, intervertebral disc herniation, IVDH, hemilaminectomy, canine, SCI, DTI, translational medicine

1. Introduction

Spinal cord injury (SCI), a devastating disease affecting the central nervous system, has a worldwide estimated incidence range in humans from 3.6 to 195.4 cases per million (Jazayeri et al. 2015). It involves individual damage of motor and visceral functions and consequently leads to detriments in quality of life and represents high economic burdens (Krueger et al. 2013). Moreover, therapeutic approaches to diminish the secondary wave damage present within the spinal cord after initial mechanical injury are still limited (Fakhoury 2015; Oliveri et al. 2014). Traditionally, the use of rodent models has been established as a highly standardized research tool for diagnostic, prognostic, and therapeutic approaches in SCI (Kim et al. 2010; Oliveri et al. 2014; Wang et al. 2014). However, induced lesions in the rodent spinal cord still evidence large discrepancies in relation to human traumatic SCI concerning pathophysiology, anatomy and histopathology (Hagg and Oudega 2006; Levine et al. 2011). Therefore, the necessity of research in large animal models that can bridge the gap between rodents and humans is evident. The dog is increasingly recognized as a large animal translational model for various pathologies of the central nervous system including multiple sclerosis, epilepsy and traumatic SCI (Jeffery et al. 2006; Levine et al. 2011; Patterson 2014; Smith and Jeffery 2006; Spitzbarth et al. 2011; Ulrich et al. 2014; van der Star et al. 2012).

Spinal cord injury caused by intervertebral disc herniation (IVDH) is one of the most common neurological conditions in dogs (Fluehmann et al. 2006). IVDH may occur when biomechanical forces are applied to a dehydrated and calcified nucleus pulposus within the intervertebral disc and consequently rupturing the dorsal aspect of the annulus fibrosus,
producing an extrusion of degenerated disc material into the vertebral canal (Bergknut et al. 2013; Jeffery et al. 2013). This spontaneously naturally occurring ventro-dorsal herniation induces a mixture of contusive and compressive forces acutely exerted to the spinal cord and therefore resembling the variability and complexity found in the human counterpart (Bock et al. 2013; Levine et al. 2011). Chondrodystrophic breeds such as Dachshunds, Pekingese, Welsh Corgi and Shi-tzu are reported to frequently suffer intervertebral disc degeneration and a genetic background has been described as an important part of its multifactorial aetiology (Mogensen et al. 2011; Priester 1976; Stigen and Christensen 1993). Depending on several factors such as the localization of the herniation, degree of compression and amount of material extruded, clinical signs may involve a wide spectrum of neurological deficits varying from mild paravertebral hyperaesthesia to paraplegia without response to nociceptive stimulus (Olby et al. 2004).

Magnetic resonance imaging (MRI) of the spinal cord remains the gold standard for the diagnosis of canine IVDH (Chang et al. 2007; Cooper et al. 2014; Levine et al. 2009); however, versatility of this technique may allow it to transcend beyond diagnostic purposes and provide valuable information concerning prognosis and early selection of patients for novel therapeutic approaches (Boekhoff et al. 2012; Kim et al. 2010). Diffusion Tensor Imaging (DTI) is a modality of MRI that enables in vivo non-invasive tissue characterization by means of water molecule diffusion (Sasiadek et al. 2012). Microarchitecture of the nervous system, particularly the white matter, permits homogeneous and direction dependent water molecule displacement with greater freedom of movement parallel to axonal bundles (Sasiadek et al. 2012). This directional dependency, also defined as anisotropy, enables DTI to infer and quantify diffusion behaviour (Vedantam et al. 2014). Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) are commonly reported indexes used for spinal cord DTI. Measurements of FA depict the degree of directionality present within a specific tissue,
and are determined by inherent tissue characteristics, for instance myelin, cellular membranes and microtubules (Beaulieu 2002; Hendrix et al. 2015). It ranges from 0 to 1, with values close to 0 meaning a random or isotropic diffusion, whereas measurements close to 1 are interpreted as highly restricted or anisotropic diffusion (Lerner et al. 2014). Furthermore, ADC represents the average magnitude of molecule displacement at any determined diffusion direction (Auriat et al. 2015; Vedantam et al. 2014).

In humans, the use of this technique has been widely described in chronic dynamic pathologies affecting the spinal cord, such as cervical spondylotic myelopathy (Banaszek et al. 2014; Guan et al. 2015). However, since time may represent a restraining factor limiting extension of MRI scans in patients with acute traumatic SCI, this field remains limitedly explored in a clinical scenario outside the rodent model. DTI measurements from different segments of canine spinal cord as well as diverse clinically applicable protocols in healthy dogs have been reported (Griffin et al. 2013; Hobert et al. 2013); nonetheless, to our knowledge, diffusion metrics from a homogeneous dog population suffering SCI have not yet been described.

The aims of this study are: (1) to compare diffusion measurements gathered from the compressed thoracolumbar spinal cord of paraplegic dogs suffering acute or subacute SCI after IVDH with the ones from spinal cords of dogs that show no clinical or diagnostic imaging evidence of thoracolumbar spinal cord disease and (2) to describe the temporal evolvement of DTI metrics in paraplegic dogs with SCI caused by IVDH which recovered voluntary motor function after surgical decompression of the spinal cord. Values were obtained from paraplegic dogs before undergoing decompressive surgery and 3 months after motor function recovery. We hypothesize that presence of herniated disc material compressing the spinal cord will alter both, direction and magnitude of diffusion and that IVDH affected dogs which regained motor function after decompressive surgery will show no
significant differences in DTI metrics in the follow up evaluation compared to unaffected dogs.

2. Materials and methods

2.1 Patients:

Nineteen private owned dogs admitted to the Small Animal Clinic of the University of Veterinary Medicine Hannover were prospectively recruited in a period between June 2013 and April 2015 with the following inclusion criteria: acute (≤7 days) or subacute (between 7 and 22 days) onset of paraplegia consistent with a T3-L3 spinal cord lesion after IVDH with presence or absence of deep pain perception (DPP), a body weight less than 20 kg and recovery of voluntary motor function within 4 weeks after decompressive surgery. DPP was tested producing a noxious stimulus, clamping the digits of the hind limbs with a haemostat. A positive reaction to this test was considered, when an obvious and reproducible behavioural response that could be interpreted as pain, such as whining, turning the head towards the origin of stimulus or attempting to bite, could be evidenced (Jeffery et al. 2016). Voluntary motor function recovery was defined as presence of pelvic limb movement evaluated with and without weight-bearing support and positive response to a noxious stimulus. Dogs with diagnosis of IVDH or spinal cord compression caudal to the intervertebral space L3/L4 or showing clinical signs compatible with a lower motor neuron lesion were excluded from the study.

Identifying the exact time point of SCI in IVDH is not always possible. Therefore, the delay of clinical signs was defined as time elapsed between owners’ recognition of the non-ambulatory state of their dog and neurological examination, and it was used to determine an acute or subacute stage of IVDH (Jeffery et al. 2016). Each patient underwent a general
physical and neurological examination, as well as diagnostic imaging consisting of radiographs of thoracic and lumbar vertebral column and MRI of the thoracolumbar spinal cord as described below. Additionally, a complete blood cell count, serum biochemistry analysis, urinalysis and examination of cerebrospinal fluid were performed to exclude several differential diagnoses. Subsequently, the spinal cord was surgically decompressed by hemilaminectomy (McKee 1992; Scott 1997). Diagnosis of IVDH was confirmed by magnetic resonance imaging and by presence of herniated intervertebral disc material during surgery. A follow-up neurological exam and MRI scan was performed approximately 3 months after motor function recovery was observed.

As controls, six dogs, 5 males and 1 female, with either orthopaedic disease or neurological signs localized outside the T3-L3 segment of the spinal cord were included (Hobert et al. 2013). Their mean age was 6.4 years (median 6.4 years; range, 1.7-12.1 years) and their mean body weight 15.6 kg (median = 11.8 kg; range, 6-30 kg). Five of the control dogs were retrospectively enrolled as their MRI sequences from a previous reported study were re-evaluated (Hobert et al. 2013). One additional dog diagnosed with idiopathic epilepsy was included in the control group. This study was performed after the approval of the German Animal Welfare authorities (Number: 33.9-42502-04-11/0661) and the written owners’ consent for each examination.

2.2 Magnetic resonance imaging:

A 3 Tesla MRI scanner (Phillips Achieva, Phillips Medical Systems, Eindhoven, The Netherlands) together with a SENSE (sensitivity encoding) - spine coil with 15 channels was used to perform the examinations. Each examination was performed under general anaesthesia. For premedication either acepromacine (0.05 mg/kg BW IM) or diazepam (0.5 mg/kg BW IV) together with levomethadone (0.2-0.6 mg/kg BW IV) was used. Anaesthesia
was induced with propofol (2 mg/kg BW IV) and maintained with isoflurane in air and oxygen. For image acquisition, dogs were placed in dorsal recumbency and at least sagittal and transversal planes of Turbo-Spin-Echo T2-weighted sequences and Echo-Planar-Imaging DWI SE sequences of the thoracolumbar spinal cord were performed.

For the acquisition of T2-weighted (T2W) sagittal images the following protocol parameters were used: TR of 3100 ms with a TE of 120 ms, slice thickness of 1.8 mm, and a slice interval of 0.2 mm. The FOV varied from 301.2 mm to 392 mm. For transversal planes of the same sequence TR varied from 4630.4 to 8418.8 ms with a TE of 120 ms, slice thickness of 2 mm, a 0.2 mm slice interval and a FOV of 190 mm. The DTI protocol consisted of a TR range of 2758.1-11668.8 ms with a TE of 70 ms, slice thickness of 2.00 mm with no slice interval, and a FOV of 214 mm. Furthermore, 32 diffusion directions were applied, number of b values = 2, low b value = 0, maximal b value = 800 sec/mm², and a voxel size of 1.98 x 2.02 x 2.00 mm.

2.3 Methods:

For DTI image processing, the software Extended MR workspace® (Version 2.6.3.4, 2012, Philips Medical Systems, the Netherlands) was used. T2W images were evaluated by board certified neurologists (AT and VS) in order to determine the localization of the IVDH for subsequent surgical approach. Additionally, these T2W images served as a baseline for anatomical land marking for the DTI. As previous reports evidenced that transversal DTI sequences minimize partial volume effects in comparison to sagittal sequences (Griffin et al. 2013), regions of interest (ROIs) were placed at the defined localizations directly in the transversal DTI sequence. In order to reduce measurement errors deriving from signals of surrounding tissues such as cerebrospinal fluid or epidural fat, the application tool “Multiple ROIs” was used to set adjacent individual voxels within the white and grey matter of the spinal cord in a transversal view. These voxels were afterwards fused in order to form a single
ROI (Fig. 1). All ROIs were placed on signal deriving from the spinal cord tissue directly dorsal to intervertebral disc spaces. Lesion epicentres were defined as localizations of spinal cord compression caused by herniated disc material in T2W sequences. ROIs were placed directly at the epicentre(s) and one vertebral body adjacent to any compression (cranially and caudally). Values FA together with ADC were gathered from each ROI. The evaluation of the DTI sequences including ROI placement as well as individual voxel placement was performed by a single examiner (AWL).

<table>
<thead>
<tr>
<th>T2W</th>
<th>FA colour maps</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Dog with IVDH" /></td>
<td><img src="image2" alt="FA colour map" /></td>
</tr>
<tr>
<td><img src="image3" alt="Control" /></td>
<td><img src="image4" alt="FA colour map" /></td>
</tr>
</tbody>
</table>

**Fig 1. Placement of regions of interest (ROI).** (A to D) T2-weighted (T2W) and FA colour maps on a transversal plane used for ROI placement on the spinal cord of a paraplegic Jack Russell terrier, 8.3 kg body weight, at the level of L1/2. (E to H) control Miniature Poodle, 10 kg body weight, at the level of L2/3. The different colours present in FA maps depict the axes of diffusion; blue in the cranio-caudal direction, red in the latero-lateral direction, and green in the dorso-ventral direction. (A and E) Spinal cord of dogs with IVDH and controls (white circle and ellipse) and herniated disc material causing compression (star) were identified in T2W images. (B and F) Signal deriving from the spinal cord was distinguished in FA maps (white circle and ellipse) and inhomogeneous and highly isotropic signal deriving from extruded material was identified (star). (C and G) Individual voxels were placed where a homogeneous cranio-caudal diffusion derived from the spinal cord was observed and herniated disc material was avoided. (D and H) Individual voxels were fused into a single ROI.
2.4 Statistical analysis:

Age and body weight of included dogs affected by IVDH and dogs belonging to the control group were compared by means of an unpaired t-test. More than one ROI was placed in lesion epicentres in dogs with spinal cord compression and distribution of herniated disc material along more than one intervertebral disc space; for statistical analysis the mean value of these measurements was calculated and applied. Additionally, DTI metrics of the control population were calculated using mean values of at least two ROIs placed in the spinal cord caudally of the twelfth thoracic vertebra and cranially of the third lumbar vertebra.

Measurements of FA and ADC values were compared between preoperative and follow up scans, as well as between dogs suffering from IVDH and controls by means of t-tests. Comparisons among the different localizations, in the lesion epicentre, cranially and caudally of the lesion, were performed using a multiple analysis of variance with effect on “localization” and a Tukey-Kramer adjustment with a significance value consideration of p<0.05. The assumption of normality was tested by means of a Kolmogorov-Smirnov test and visual assessment of qq-plots of model residuals. Right skewed distributed data was log-transformed prior to calculation; afterwards, results were retransformed to normal scale for description (geometric mean and geometric standard deviation depicted). For this purpose, the commercially available software SAS®, version 9.2 (SAS Institute, Cary, NC, USA) and GraphPad Prism® (version 5, GraphPad Software, CA, USA) were used for the statistical calculations and graphic elaboration, respectively.
3. Results

3.1 Dogs

Nineteen dogs, 9 females and 10 males, suffering from SCI caused by IVDH were included. The patients had a mean age of 5.5 years (median = 5.1 years; range, 2.2-13.1 years) and a mean body weight of 9.7 kg (median = 8.3 kg; range, 3.8-19.6 kg). Dachshunds (n = 7) and mixed-breed dogs (n = 5) were the most common. Moreover, two Jack Russell terriers, two Shih Tzu, and one individual of each of the following breeds were recruited: Havanese, small Munsterlander pointer, and French bulldog. No significant difference was found in age or body weight between affected dogs and controls (Table 1). The mean time between onset of non-ambulatory status and preoperative MRI examination was 3 days (median = 1 day; range 0-22 days). The most commonly affected intervertebral disk spaces were Th12/13 and Th13/L1. Neurological examination revealed that all patients were paraplegic, of which 14 showed a response to nociceptive stimulation and 5 dogs showed no presence of DPP in the pelvic limbs (Table 2). All paraplegic dogs underwent surgical decompression of the spinal cord immediately after MRI and regained motor function within 4 weeks after surgery. Follow up MRI examination was performed at a mean time of 15.6 weeks after pre-operative scan (median: 15.5 weeks; range 12-20 weeks) and at this time point, all dogs were able to walk, one of them with support. In one dog with clinical improvement the follow up scan could not be performed.

Table 1. Comparison of age and weight between groups

<table>
<thead>
<tr>
<th></th>
<th>Dogs with IVDH (n=19)</th>
<th>Controls (n=6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (± SD)</td>
<td>5.5 (± 2.888)</td>
<td>6.4 (± 3.578)</td>
<td>0.5229</td>
</tr>
<tr>
<td>Mean body weight (± SD)</td>
<td>9.7 (± 4.268)</td>
<td>15.6 (± 10.241)</td>
<td>0.0515</td>
</tr>
</tbody>
</table>

IVDH, intervertebral disc herniation; SD, standard deviation
### Table 2. Patient Characteristics

<table>
<thead>
<tr>
<th>Dog</th>
<th>Breed</th>
<th>Gender</th>
<th>Age (y)</th>
<th>Body weight (Kg)</th>
<th>Delay of clinical signs</th>
<th>Localization of IVDH</th>
<th>DPP</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Mixed-breed</td>
<td>M</td>
<td>7.0</td>
<td>12.8</td>
<td>0</td>
<td>Th13/L1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Dachshund</td>
<td>F</td>
<td>5.3</td>
<td>5.6</td>
<td>0</td>
<td>Th12/13</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Jack Russell Terrier</td>
<td>M</td>
<td>4.4</td>
<td>7.4</td>
<td>1</td>
<td>Th12/13</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Shih Tzu</td>
<td>F</td>
<td>2.8</td>
<td>5.6</td>
<td>2</td>
<td>Th12/13</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Dachshund</td>
<td>F</td>
<td>6.0</td>
<td>8.1</td>
<td>22</td>
<td>Th11/12</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Dachshund</td>
<td>M</td>
<td>9.7</td>
<td>14.8</td>
<td>0</td>
<td>L1/2</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>French Bulldog</td>
<td>F</td>
<td>2.7</td>
<td>10.9</td>
<td>1</td>
<td>Th12/13</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Mixed-breed</td>
<td>M</td>
<td>3.8</td>
<td>6.4</td>
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<td>Th13/L1</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Small Munsterlander pointer</td>
<td>M</td>
<td>2.7</td>
<td>13.5</td>
<td>1</td>
<td>Th12/13</td>
<td>+</td>
</tr>
<tr>
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<td>Dachshund</td>
<td>F</td>
<td>6.2</td>
<td>8.6</td>
<td>1</td>
<td>Th12/13</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Jack Russell Terrier</td>
<td>M</td>
<td>4.6</td>
<td>8.3</td>
<td>0</td>
<td>L1/2</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Mixed-breed</td>
<td>F</td>
<td>10.1</td>
<td>19.6</td>
<td>1</td>
<td>Th13/L1</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Mixed-breed</td>
<td>M</td>
<td>2.2</td>
<td>3.8</td>
<td>0</td>
<td>L1/2</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Dachshund</td>
<td>F</td>
<td>4.1</td>
<td>6.7</td>
<td>5</td>
<td>Th11/12</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Dachshund</td>
<td>F</td>
<td>5.3</td>
<td>8.1</td>
<td>10</td>
<td>Th11/12</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>Mixed-breed</td>
<td>M</td>
<td>6.6</td>
<td>17.7</td>
<td>0</td>
<td>L1/2</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>Havanese</td>
<td>M</td>
<td>2.6</td>
<td>6.4</td>
<td>4</td>
<td>Th13/L1</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>Shih Tzu</td>
<td>M</td>
<td>13.1</td>
<td>9.1</td>
<td>2</td>
<td>Th11/12</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>Dachshund</td>
<td>F</td>
<td>5.1</td>
<td>10.8</td>
<td>6</td>
<td>Th11/12</td>
<td>-</td>
</tr>
</tbody>
</table>

IVDH, intervertebral disc herniation; DPP, deep pain perception; M, male; F, female; Th, thoracic vertebra; L, lumbar vertebra; +, presence of DPP; -, absence of DPP.
3.2 Fractional anisotropy

Fractional anisotropy depicts the directional restriction of diffusion of water molecules (Hendrix et al. 2015; Sasiadek et al. 2012). Before decompressive surgery, values of FA at the site of the lesion epicentre in paraplegic dogs after IVDH were higher than in the controls (p=0.0039). At the same time point, FA values at epicentres were significantly higher compared to the values one vertebral body caudally (Fig. 2). Furthermore, temporal evolvement of FA could be evaluated by comparing the values collected before hemilaminectomy with the ones measured at follow up 3 months after functional motor recovery. Before decompression, FA was significantly higher at the epicentres as well as cranial and caudal to the lesion compared to follow up evaluation (Table 3). Three months after functional motor recovery, FA showed no statistical difference when compared with the control group (Fig. 2).

![Fig 2. Distribution of FA values. Tukey boxplots depicting the distribution of FA at each localization before (A) and 3 months after functional motor recovery (B). Values at epicentres showed significant increases compared to controls and perilesional values measured caudal to the epicentre.](image)
### Table 3. Temporal evolvement of DTI metrics after spinal cord decompression.

<table>
<thead>
<tr>
<th>Diffusion metrics</th>
<th>Segment</th>
<th>State before surgery (n=19)</th>
<th>Follow up (n=18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA; Median ± SD</td>
<td>Cranial</td>
<td>0.715 (±0.107)</td>
<td>0.604 (±0.104)</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Epicentres</td>
<td>0.769 (±0.064)</td>
<td>0.571 (±0.064)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Caudal</td>
<td>0.667 (±0.087)</td>
<td>0.581 (±0.096)</td>
<td>0.0008</td>
</tr>
<tr>
<td>ADC (10³ mm²/sec); Median ± geometric SD</td>
<td>Cranial</td>
<td>0.935 (+0.298; -0.226)</td>
<td>1.136 (+0.498; -0.346)</td>
<td>0.0029</td>
</tr>
<tr>
<td></td>
<td>Epicentres</td>
<td>0.783 (+0.198; -0.158)</td>
<td>1.134 (+0.419; -0.306)</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Caudal</td>
<td>0.953 (+0.204; -0.168)</td>
<td>1.142 (+0.490; -0.343)</td>
<td>0.0368</td>
</tr>
</tbody>
</table>

Epicentres: ROIs placed in spinal cord compressed by herniated nucleus pulposus material, directly above the respective intervertebral disc space. Cranially: ROIs placed in spinal cord one vertebral body cranially to epicentres. Caudally: ROIs placed in spinal cord one vertebral body caudal to epicentres. Abbreviations: DTI = diffusion tensor imaging, FA = fractional anisotropy, ADC = apparent diffusion coefficient, SD = standard deviation.

#### 3.3 Apparent diffusion coefficient

The mean magnitude of water molecule diffusion within axonal bundles is described by the ADC value (Sasiadek et al. 2012). In the acute stage before decompressive surgery, ADC values were lower in the epicentres compared to ROIs set one vertebral body cranially or caudally (p=0.0235 and p=0.0100, respectively; Fig. 3). Moreover, ADC values evaluated in all three spinal cord segments in dogs with IVDH were significantly lower than control values (Fig. 3). Temporal evolvement of ADC values could be evidenced with t-tests performed between pre-operative measurements and follow up scans. ADC values were significantly lower in the compressed spinal cord than in the follow up status at the epicentre as well as
perilesional (p<0.0002 for epicentres; p=0.0029 cranially; p=0.0368 caudally; Table 3). Additionally, no significant differences could be found between ADC values at the time of follow up examinations and controls (Fig. 3).

**Fig 3. Distribution of ADC values.** Tukey boxplots depicting the distribution of ADC at each localization before (A) and at follow up scan 3 months after functional motor recovery (B). Before decompressive surgery, values gathered from the different localizations were significantly lower than that of controls. Epicentres displayed lower values than values cranially and caudally.
4. Discussion

Clinical approach for acute traumatic SCI faces substantial challenges including the fact that current techniques to assess severity and recovery are non-quantitative (Krishna et al. 2014). DTI is a promising non-invasive tool for microstructural evaluation of the spinal cord that could be implemented during the recovery phase. Furthermore, canine SCI has been increasingly recognized as a translational model for human traumatic SCI (Granger et al. 2012; Jeffery et al. 2006; Levine et al. 2011; Smith and Jeffery 2006). In this prospectively designed study, temporal evolvement of DTI values measured in the spinal cord of paraplegic dogs regaining motor function subsequent to decompressive surgery is described.

The population of dogs presented and treated for IVDH match with previous reports, being mostly middle-aged dogs of chondrodystrophic breeds (Bergknut et al. 2012; Hansen 1952; Priester 1976). Furthermore, localization of disc herniation within the vertebral column occurred at the most commonly reported sites (Brisson et al. 2004; Hansen 1951; Olby et al. 2004). The vertebral thoracolumbar junction has been reported to be a common segment for occurrence of IVDH as the presence of intercapital ligaments in the mid-thoracic vertebral column may partially prevent intervertebral discs to herniate dorsally or dorsolaterally (Hansen 1951).

DTI was introduced as an MRI sequence by Basser and colleagues in 1994, and since then, medical research concerning water molecule diffusivity, directionality and fibre tractography of brain and most recently spinal cord pathologies has become a focus of interest (Basser et al. 1994; Martin et al. 2016; Vedantam et al. 2014). Diffusion metrics within the lesioned spinal cord have been widely described in humans presenting chronic, traumatic or dynamic compressive, cervical spinal cord lesions (Li et al. 2016). However, only few reports concerning the acute state of SCI are available, describing different methodologies and
inconsistent results among each other (Cheran et al. 2011; Facon et al. 2005; Shanmuganathan et al. 2008; Vedantam et al. 2013). This small number of reports can be presumably explained by the urgent necessity to surgically decompress the spinal cord or stabilize the vertebral column of these patients and therefore favouring diagnostic approaches such as short time MRI scans, including conventional sequences only.

Both, FA and ADC, have been measured in laboratory models of traumatic SCI in rodents (Jirjis et al. 2013; Kelley et al. 2014; Kim et al. 2010; Vedantam et al. 2014; Wang et al. 2014). In such models FA values decreased at the site of the lesion epicentre directly after the injury was initiated and mildly increased again, tending to values of the control group weeks after the injury (Wang et al. 2014). Reported ADC values after acute SCI have been rather contradictory, being described either as increased or decreased (Li et al. 2015; Wang et al. 2014). Interestingly, the results of the current study display increased FA measures in paraplegic dogs acutely affected with SCI at the time point before undergoing surgical decompression. This difference to rodent studies can be explained considering the pathophysiological differences between both models. SCI in rodents is homogeneously induced by a defined weight drop contusion, hemi-transection or total transection of a non-compressed spinal cord after performing a dorsal vertebral laminectomy (Hu et al. 2010; Jirjis et al. 2013; Wang et al. 2014), whereas canine SCI in the current study is caused by a spontaneous, naturally-occurring herniation of degenerated nucleus pulposus content that exerts first a contusive force on the spinal cord ventrally and afterwards a continuously compressive force produced by the herniated disc material present in the vertebral canal (Jeffery et al. 2013; Levine et al. 2011). Acute reduction of vertebral canal diameter and consequently compression of white matter tracts may have an important effect in directionality of diffusion, making it more anisotropic at this time point. Moreover, it has been proposed that FA values are more dependent on changes of cellular membranes than on
myelin sheaths (Vedantam et al. 2014). Since the most predominant histopathological findings present at the lesion epicentre of acute and subacute injured canine spinal cord after IVDH are axonal swelling and myelin sheath degeneration, accompanied with a variable level of oedema and haemorrhage (Bock et al. 2013; Smith and Jeffery 2006), our findings suggest that increases of FA may potentially represent a marker for cytotoxic oedema, as it is presumed to occur within corticospinal tracts and corpus callosum of humans suffering acute traumatic brain injury (Henry et al. 2011; Wilde et al. 2008).

Furthermore, diffusivity changes, depicted by decreased ADC, were found in all evaluated segments of the spinal cord in dogs before undergoing decompressive surgery compared to control values. Intra-axonal ultrastructural changes such as disarrangement of axoplasmic neurofilaments and mitochondrial accumulation have been evidenced at lesion epicentre and even at distant segments away from the compression site (Bock et al. 2013; Smith and Jeffery 2006). Therefore, low perilesional diffusion magnitude found in dogs with SCI being directionally independent (Shanmuganathan et al. 2008), seems to be an indicator of such intracellular damage. Additionally, significantly lower ADC values at epicentre compared to ADC values of the spinal cord one vertebral body cranially and caudally suggest a complimentary distorted diffusivity caused by mechanical compression and permanent deformation exerted by the extruded disc material on the spinal cord at the time of the preoperative MRI scan.

At follow up examination 3 months after recovery of motor function, both, FA and ADC values showed no differences when compared to control individuals, indicating neither restriction of magnitude or direction of water molecule diffusion and therefore revealing accordance between diffusion metric tendency to normality and motor function recovery of the dogs. This temporal change after prompt surgical intervention may indicate a relative
conservation of tissue architecture as a consequence of moderate to severe injury, since the majority of paraplegic dogs showed a preserved deep pain perception in clinical-neurological examinations. Furthermore, timely surgical decompression could have led to effective reperfusion of spinal cord parenchyma, thereby avoiding possible worsening of clinical signs. Additionally, these findings suggest that complex intrinsic reparatory mechanisms take place within the canine spinal cord days after SCI caused by IVDH. Examples of such mechanisms are expression of Growth Associated Factor-43 (GAP-43) as indicator of axonal regeneration and remyelination accomplished by Schwann cells and oligodendrocytes playing an important role in microarchitecture preservation and remodelling (Bock et al. 2013; Smith and Jeffery 2006).

A prospectively recruited relatively homogeneous population of dogs with SCI weighing less than 20 kg reduces anatomical and outcome variabilities as a previous study reported weight as a delay factor for motor function recovery (Olby et al. 2003). Additionally, all DTI sequences were evaluated, as well as all ROIs were placed by a single examiner (AWL) to avoid variations caused by different observers.

As all dogs recruited in this study were still alive at the time of its completion, the lack of histopathological and immunohistochemical studies of epicentres and perilesional spinal cord segments represents a limitation. However, this reflects similar conditions of studies in human beings. Spatial resolution of the lower thoracic spinal cord in DTI sequences remains a challenge in the human spinal cord with a larger diameter when compared with the canine cord (Ellingson et al. 2008; Levine et al. 2009). Therefore, a clear distinction between white and grey matter, as well as visualization or evaluation of diffusion metrics of individual funiculi using clinical applicable protocols in the canine spinal cord is still beyond the study’s scope. For this reason, we performed ROI placement of individual voxels including both, grey
and white matter as formerly reported (Griffin et al. 2013; Hobert et al. 2013; Santarelli et al. 2010). Additionally, delay and severity of clinical signs varied between cases. Rather than being considered as a limitation, this feature should emphasize the appropriateness of spontaneous, naturally occurring canine SCI as a translational model for evaluation of traumatic SCI, as it encompasses similar heterogeneity of its human counterpart (Dvorak et al. 2015; Jeffery et al. 2006).

Establishing novel therapeutic interventions for patients suffering SCI is challenging and necessary. Therefore, research evaluating minimal- or non-invasive in vivo techniques, such as DTI, that could accompany conventional diagnostic tools is mandatory to monitor ultrastructural changes in the recovery phase. DTI encompasses promising features for better understanding clinical and pathophysiological mechanisms of several spinal cord diseases (Budrewicz et al. 2016; Guan et al. 2015; Koskinen et al. 2013; Oh et al. 2013). However its implementation in routine spinal cord MRI scan protocols for acute SCI as a prognostic indicator has still to be elucidated (Martin et al. 2016). As feasibility of monitoring temporal evolvement of diffusion metrics in paraplegic dogs with motor function recovery after surgical decompression was proven, the present study can set a basis for future research regarding prognostic value determination or follow-up studies to evaluate effects of novel therapeutic interventions on a microstructural level. As hypothesised, distortion in water molecule diffusion present in paraplegic dogs shortly after losing ambulation is no longer evident 3 months after recovering voluntary motor function. To the authors’ knowledge, this is the first report of temporal diffusion metrics evolvement in a homogeneous population of dogs with IVDH representing a spontaneous, naturally occurring model for human SCI. In summary, an increase of FA and decrease of ADC was found to be more evident at the epicentre of the acutely compressed spinal cord; such metrics showed no differences with control values 3 months subsequent to recovery of voluntary motor function. The findings
suggest that the compressive component during IVDH may play an important role in diffusion dynamics during acute and subacute SCI as well as that FA and ADC could be potential markers for cytotoxic oedema and intraaxonal ultrastructural alterations found in canine SCI, respectively.

5. Acknowledgements

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6. Conflicts of interest

Authors disclose no conflicts of interest.
7. References


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Chapter 3: Temporal Evolvement of DTI Metrics in Paraplegic Dogs


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4. Chapter 4: Evaluation of acute and chronic SCI using DTI

A. Wang-Leandro: performed acquisition of images, generated and analysed the data, and wrote the manuscript.

M. K. Hobert: acquisition of images, advice concerning methods.

N. Alisauskaite: analysed partly data of chronic patients.

P. Dziallas: performed acquisition of images.

K. Rohn: performed statistical analysis.

V. M. Stein: was involved in the concept and design of the study and critically revised the manuscript, performed partly surgical approaches.

A. Tipold: was involved in designing of the study, discussing analysis of the data and critically revised the manuscript.

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Running title: Comparison of acute and chronic SCI using DTI

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Chapter 4: Evaluation of acute and chronic SCI using DTI

Abstract

Canine spinal cord injury (SCI) occurs spontaneously and is characterised by a contusive-compressive lesion, mimicking conditions in human traumatic SCI. Diffusion tensor imaging (DTI) allows in vivo quantitative evaluation of microstructural integrity of the spinal cord. Our aim was to describe DTI metrics obtained from the spinal cord of dogs with severe acute or chronic SCI and to correlate DTI values with lesion extension of SCI measured in conventional T2-weighted (T2W) sequences. Forty-seven paraplegic dogs with thoracolumbar SCI, 32 with acute and 15 with chronic SCI, and 6 control dogs were included. T2W and DTI sequences of the thoracolumbar spinal cord were performed. Values of fractional anisotropy (FA) and apparent diffusion coefficient (ADC) were obtained from the epicentre of the lesion and one spinal cord segment cranially and caudally and compared between groups. Lesion extension of SCI was measured in T2W sequences. Pearson’s correlation coefficient was calculated between DTI metrics and lesion extension.

Dogs with chronic SCI revealed a lower FA and higher ADC compared to dogs with acute SCI (p<0.0001 for both values at epicentres, cranially and caudally). In dogs with acute SCI, FA values were increased (p=0.0065) and ADC values were decreased (p=0.0099) at epicentres compared to controls. FA values obtained at lesion epicentre and one spinal segment cranially in dogs with chronic SCI correlated with extension of lesion (r=0.5517 for epicentres and r=0.6810 segment cranially). Using DTI, microstructural differences between acute and chronic stages of SCI in naturally occurring canine SCI could be detected and correlations between T2W and DTI sequences were found in chronic SCI. These findings emphasize the role of canine SCI as a large animal translational model for human SCI.

Key Words

DTI, SCI, animal model, translational medicine, canine
1. Introduction

Traumatic spinal cord injury (SCI) is a frequently occurring neurological condition that can lead to permanent loss of sensorimotor and visceral function (Silva et al. 2014). Initial cellular destruction caused by direct mechanical damage is defined as primary injury (Silva et al. 2014). Seconds after primary injury occurs, dynamic and complex cellular responses take place, including cytokine production, excitotoxicity, inflammatory reactions and free radical release associated with variable extension of oedema and haemorrhage (Silva et al. 2014; Smith and Jeffery 2006; Witiw and Fehlings 2015). Further intrinsic de- and regenerative response mechanisms to injury end up with a consolidation of an astrocyte mediated glial scar (Bock et al. 2013; Hu et al. 2010; Smith and Jeffery 2006). Such cascade of dynamic events is known as the secondary wave damage (Oyinbo 2011; Silva et al. 2014). Current assessment of severity and extension of SCI encompasses evaluation of compression and intramedullary hyperintense signal in conventional T2-weighted (T2W) magnetic resonance imaging (MRI; Becerra et al. 1995; Bodley 2002; Lammertse et al. 2007; McDonald and Sadowsky 2002; Yamashita et al. 1990). Treatment for traumatic SCI includes decompression of the spinal cord and stabilization of the vertebral column (Fehlings and Perrin 2005); nonetheless, specific therapy targeted to mitigate the effects of the secondary wave damage remains limited (Granger et al. 2014; Raspa et al. 2016; Silva et al. 2014).

Several animal models have been used to reproduce SCI and contribute to a better understanding of pathophysiological processes that take place during different temporal stages (Granger et al. 2012; Hu et al. 2010; Jiang et al. 2016; Ma et al. 2016; Streijger et al. 2016; Szarek et al. 2016). The most commonly applied is the rodent model, as laboratory conditions enable low variability (Silva et al. 2014); however, the necessity of a large animal model has led to an increased acknowledgement of the dog as a well-established, spontaneous, and
naturally occurring model for human traumatic SCI (Boekhoff et al. 2012; Jeffery et al. 2006; Levine et al. 2011). SCI in dogs is most commonly caused by intervertebral disc herniation (IVDH) and external blunt traumas (Fluehmann et al. 2006; Priester 1976). Canine SCI is characterized by contusive - compressive forces concurrently damaging the spinal cord (Jeffery et al. 2013; Jeffery et al. 2006). The pathogenesis implies a wide extent of variability concerning severity of clinical signs, localization, and degree of spinal cord compression (Jeffery et al. 2006; Olby et al. 2004), resembling human traumatic SCI and representing a valuable opportunity to bridge the gap between rodents and humans (Bock et al. 2013; Levine et al. 2011; Olby et al. 2004).

Diffusion tensor imaging (DTI) enables \textit{in vivo} and non-invasive assessment of white matter tracts of the spinal cord (Martin et al. 2016). Microstructural barriers such as myelin and cellular membranes of axonal tracts facilitate a homogeneous and directionally dependent diffusion (Hendrix et al. 2015). Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) are the most commonly evaluated diffusion metrics (Guan et al. 2015). FA is expressed as a unitless numerical scale, where FA equal to zero indicates a directionally unrestricted or equally restricted diffusion, whereas FA equal to one represents a completely restricted diffusion along a single axis (Chagawa et al. 2015; Jellison et al. 2004; Lerner et al. 2014). While FA expresses the direction of diffusion, ADC reflects the capacity and subsequently the magnitude of water molecules to diffuse in any given preferential direction (Chagawa et al. 2015; Guan et al. 2015; Li et al. 2016; Soares et al. 2013).

Assessment of human SCI using DTI has been particularly performed for the chronic stage of the disease (Martin et al. 2016; Wheeler-Kingshott et al. 2014), consequently enhancing the use of animal models for studies concerning the acute phase (Kelley et al. 2014; Kim et al. 2010; Li et al. 2015; Yin et al. 2010). Little is known about \textit{in vivo} diffusion behaviour after
severe SCI comparing acute and chronic stages, being mostly reported in rodents showing some degree of motor function recovery and therefore omitting a population of animals having an unfavourable prognosis (Jirjis et al. 2013; Wang et al. 2014; Zhao et al. 2016). Evaluation and description of the spinal cord in the chronic phase of severe injury by means of DTI is essential, as for these individuals novel therapy implementation is urgently needed (Granger et al. 2012; Raspa et al. 2016; Sarmento et al. 2014).

The aim of this study is to describe diffusion metrics obtained from the spinal cord of paraplegic dogs with acute or chronic SCI using a clinically applicable DTI protocol and to correlate DTI values with lesion extension of SCI measured in conventional T2W sequences. We hypothesize, (1) that DTI is capable of detecting microstructural differences between acute and chronic SCI; and (2) that values obtained from the spinal cord of paraplegic dogs suffering chronic SCI will show a more isotropic diffusion and longer extension of lesion in T2W sequences compared to paraplegic dogs with acute SCI.

2. Materials and Methods

2.1 Dog Population

For the present study, paraplegic dogs admitted to the Department of Small Animal Medicine and Surgery, University of Veterinary Medicine Hannover were prospectively recruited. Dogs with paraplegia due to SCI with or without presence of deep pain perception (DPP), a neuroanatomical localization of the spinal cord lesion in the T3 to L3 segments and a body weight less than 20 kg were included. Further tests comprised physical and neurological examinations, radiographs of the vertebral column, blood cell count and serum biochemistry analysis, urinalysis, MRI of the thoracolumbar segment of the spinal cord, and CSF examination. Intact DPP was defined as a targeted behavioural response (such as whining,
turning the head towards the stimulus or attempting to bite) after clamping the distal phalanges of the hind limbs with forceps (Gorney et al. 2016; Jeffery et al. 2016).

SCI in dogs occurs spontaneously. Time of initial SCI had therefore to be defined as the time point of non-ambulatory state first noticed by the owners (Jeffery et al. 2016). Paraplegic dogs were assigned into two different groups with regard to the time point of neurological and MRI examinations in the clinic: the acute SCI group (≤7 days) and the chronic SCI group (≥28 days; Griffin et al. 2015; Hu et al. 2010; Levine et al. 2009). Moreover, MRI sequences from six previously reported dogs, 5 males and 1 female, with either orthopaedic disease or neurological signs localized outside the T3-L3 segments of the spinal cord were included as controls (Hobert et al. 2013). Their mean age was 6.4 years (median 6.5 years; range 1.7-12.1 years) and their mean body weight 15.6 kg (median 11.8 kg; range 6-30 kg). This study was performed according to the German animal welfare statutes (Number: 33.9-42502-04-11/0661) and the written consent of the dog owners for each examination.

2.2 Image acquisition

MRI scans were performed under general anaesthesia and assisted ventilation and dogs were positioned in dorsal recumbency to avoid movement artefacts (Griffin et al. 2013). A sensitivity-encoding (SENSE) spinal coil with 15 channels was used. Transversal and sagittal Turbo-Spin-Echo T2W as well as single-shot Echo-Planar-Imaging DWI SE sequences of the thoracolumbar spinal cord were performed in all dogs using a 3T MRI scanner (Phillips Achieva, Eindhoven, The Netherlands).

Protocol used for acquisition of sagittal T2W images consisted of repetition time (TR) between 3100 and 4786.4 ms, 120 ms echo time (ET), field of view (FOV) between 260.7 and 392.0 mm, a slice thickness of 1.8 mm and space between slices and a space between slices of 0.2 mm. Furthermore, the transversal T2W sequences were acquired using TR between
5472.2 and 9681.7 ms, ET of 120 ms, FOV of 190 mm, slice thickness of 2 mm and space between slices of 0.2 mm.

Regarding DTI sequences, the protocol had an acquisition matrix of 108 x 98, FOV of 214 mm and 70 ms ET. TR varied between 2758 and 11713 ms depending on slice number adapted according to the dog’s size. Thirty-two diffusion directions were applied (low b value = 0, maximal b value = 800 sec/mm²). Furthermore, reconstructed voxel size was determined for 1.67 x 1.65 x 2.00 mm, slice thickness was fixed in 2 mm and no space was left between slices. Phase correction was automatically applied during acquisition of DTI sequences and SPIR fat suppression was implemented in order to diminish interference from epidural fat. Moreover, dynamic stabilization was used at acquisition time point to improve image consistency and for motion correction. Diffusion registration was applied for eddy-current distortion corrections as previously reported (Chun et al. 2016; Hobert et al. 2013; Kwon et al. 2011).

2.3 Image processing

T2W sequences were examined by at least one board certified neurologist (AT and/or VMS) in order to determine localization of lesion and presence of spinal cord compression. Moreover, assessment of lesion extension of SCI was performed in sagittal T2W planes. Lesion extension of the spinal cord in T2W sequences was defined as segments presenting herniated disc material causing compression of the spinal cord and associated intramedullary hyperintense signal (Boekhoff et al. 2012; Ito et al. 2005). Extension of spinal cord lesion was assessed in sagittal T2W images. Lengths of hyperintense signal and spinal cord compression were measured in millimetres and expressed as a ratio in relation to the vertebral body length of the second lumbar vertebra as previously described and defined as T2W lesion extension ratio (T2W-LER; Boekhoff et al. 2012; Ito et al. 2005). Evaluation of T2W-LER was
performed using the commercial available software easyVET® (Version 8.0.0.03/R3, 2015, Isernhagen, Germany).

**Fig. 1:** Sagittal plane T2-weighted (T2W) images from the spinal cord of a male Jack Russell Terrier, age 4.4 years and body weight 7.4 kg with an acute spinal cord injury (SCI) after intervertebral disc herniation (IVDH) at level of Th12/13 (A) and a male Dachshund, age 4.8 years and body weight 8.8 kg affected by a chronic SCI caused by IVDH at Th11/12 (B). Length of vertebral body L2 and T2W lesion extension are shown with green lines.

Definition of the regions of interest (ROI) in DTI sequences was performed using the Extended MR workspace software (Version 2.6.3.4, 2012, Philips Medical Systems, The Netherlands). For this purpose, T2W images were placed over FA maps serving as template for determination and positioning of the ROIs. In dogs with SCI, values of FA and ADC were obtained from ROIs placed within signal deriving from spinal cord at epicentre(s), as well as one spinal segment cranially and caudally. In dogs presented with an acute SCI, epicentres were defined as segments of spinal cord with contusion and/or compression caused by IVDH or vertebral fracture; whereas in dogs presented with chronic SCI, epicentres were defined as spinal cord segments with compression or evidence of previous surgical decompression suggesting the initial localization of SCI. In FA maps, signal deriving from spinal cord parenchyma above intervertebral disc spaces was first identified. Herniated disc material and fluid filled cavitations were identified when present and excluded from measurements; therefore, only signal from spinal cord parenchyma was taken into consideration for ROI
placement (Fig. 2). Individual voxels were first placed within the white and grey matter of the spinal cord using the application tool “Multiple ROIs” avoiding signals representing cerebrospinal fluid (CSF) and epidural fat. Voxels were set in transversal FA maps to minimize partial volume effects (Griffin et al. 2013).

Fig. 2: Transversal planes of T2-weighted (T2W) and diffusion tensor imaging - fractional anisotropy (FA) maps of the spinal cord from (A) a male dachshund at L1/2, age 9.7 years and body weight 14.8 kg, with an acute spinal cord injury (SCI) after intervertebral disc herniation (IVDH) and (B) a female French bulldog, age 2.8 years and body weight 13 kg, with a chronic SCI at L2/3. Colour coding of FA maps denote water diffusion in cranio-caudal axis in blue, in latero-lateral axis in red, and in ventro-dorsal axis in green. The star shows disc material compressing the spinal cord, which is represented as inhomogeneous mostly hypointense in comparison to surrounding spinal cord tissue in T2W sequences and inhomogeneous diffusion direction together with signal voidance in FA maps. Arrows point at cavitations within the spinal cord noted as hyperintense signal in comparison to surrounding spinal cord tissue in T2W images and signal void in FA maps, indicating unrestricted diffusion direction. Area considered for placement of regions of interest (ROIs) is delimitated by a white contour.
2.4 Statistical Analysis

FA and ADC values obtained from dogs that required positioning of more than one ROI at the lesion epicentre were reported as mean values. Similarly, in control dogs, mean values of DTI metrics obtained from at least two localizations between T12 and L3 segments were calculated. Assumption of normal distribution of DTI values was tested by means of Kolmogorov-Smirnov test as well as visual evaluation of qq-plots of model residuals. Moreover, a one-way variance analysis was performed to compare DTI metrics between groups at each independent spinal localization. Furthermore, covariance calculations to assess influence of either age or body weight over FA and ADC values were additionally implemented for each variance analysis. Student’s t-tests were performed to compare T2W-LER between acute and chronic SCI affected dogs. Correlations between DTI values and T2W-LER obtained from paraplegic dogs were determined by means of a Pearson’s correlation coefficient. Significance level was set at p<0.05, statistics were performed using SAS® software, version 9.2 (SAS Institute, Cary, NC, USA) and graphics were generated utilizing the commercial software GraphPad Prism® (version 5, GraphPad Software, CA, USA).
3. Results

3.1 Population

A total of 47 paraplegic dogs with thoracolumbar SCI, 28 males and 19 females, fulfilled the inclusion criteria. Mean age was 5.7 years (median 4.8 years, range 2-16 years) and mean body weight was 9.1 kg (median 8.8 kg, range 3.8-19.6 kg; detailed information of patients is contained in Table 1). Thirty-two dogs were assigned to the group of acute SCI (time point of non-ambulatory state first noticed by the owners to examination ≤ 7 days; mean 1.4 days, median 1 day, range 0 – 7 days), whereas 15 dogs were assigned to the chronic SCI group (time point of non-ambulatory state first noticed by the owners to examination ≥ 28 days; mean 167.1 days, median 136 days, range 53 – 365 days). In three dogs belonging to the chronic SCI group, it was not possible to determine the exact time point of initial trauma precisely as dogs came from animal shelters. Furthermore, the age could not be determined in one of these dogs for the same reason. All dogs with an acute SCI were diagnosed with IVDH, with the exception of one dog with a vertebral fracture following exogenous trauma. Most dogs with chronic SCI had evidence of former decompressive surgery due to IVDH.
Table 1. Characteristics of paraplegic dogs at the time point of inclusion in the study.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Breed</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Body weight (Kg)</th>
<th>Time from onset of non-ambulatory status to MRI examination (days)</th>
<th>DPP</th>
<th>Localization of epicentre(s)</th>
<th>T2W-LER</th>
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<td></td>
<td>Acute SCI (≤ 7days)</td>
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<tr>
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<td>M</td>
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<td>F</td>
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<td>9</td>
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<td>M</td>
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<td>+</td>
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<td>32</td>
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**Chronic SCI (≥28 days)**

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<th>Age (months)</th>
<th>Body Weight (kg)</th>
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<th>Th13/L1</th>
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<td>53</td>
<td>-</td>
<td>Th13/L1</td>
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<td>L1/L2</td>
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<td>-</td>
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<td>3.2</td>
<td>10.3</td>
<td>109</td>
<td>-</td>
<td>L1/2; L2/3</td>
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</tbody>
</table>

Abbreviations: MRI, magnetic resonance imaging; DPP, deep pain perception; +, presence of DPP; -, absence DPP; SCI, spinal cord injury; T2W-LER, T2-weighted lesion extension ratio; M, male; F, female; IVDH, intervertebral disc herniation; Th, thoracic vertebra; L, lumbar vertebra; JRT, Jack Russell Terrier; WHWT, West Highland White Terrier.
3.2 Fractional anisotropy

Measured FA values are summarized in table 2. Comparison of dogs with acute or chronic SCI revealed a highly significant difference at each spinal cord localization in respect to the lesion (p<0.0001 at epicentres, cranially and caudally; Fig. 3). Moreover, FA values from the group of dogs with chronic SCI dogs were significantly lower when compared to control dogs (p<0.0001 at epicentres and caudally; p=0.0002 cranially; Fig. 3). At the level of epicentres, values measured from the group of dogs with acute SCI were higher than of control dogs (p=0.0065); furthermore, no difference was found perilesional between the group with acute SCI and controls (p=0.2054 cranially and p=0.4968 caudally to epicentre; Fig. 3). Covariance analysis performed between chronic SCI, acute SCI and control groups revealed no influence of body weight (p=0.8938 at epicentres, p=0.8956 cranially and p=0.8816 caudally) or age (p=0.5489 at epicentres, p=0.7151 cranially and p=0.7734 caudally) among any of the groups on the variance analysis.
Chapter 4: Evaluation of acute and chronic SCI using DTI

Fig. 3: Tukey boxplots depicting distribution of fractional anisotropy (FA) measurements obtained from paraplegic dogs after acute or chronic spinal cord injury (SCI) at each evaluated segment. Second quartile, median, and third quartile of FA obtained from control dogs are depicted in the background as a grey area. Significance levels between acute and chronic SCI affected dogs are shown with stars (*), whereas significance level between acute or chronic SCI affected dogs and controls are depicted with numerical signs (#).

3.3 Apparent diffusion coefficient

ADC values are summarized in table 2. Paraplegic dogs suffering from acute SCI had significantly lower ADC values at each localization compared to those with chronic SCI (p<0.0001 for epicentres, cranially and caudally; Fig. 4). Values obtained from epicentres in dogs suffering from acute SCI differed from control values, being significantly lower (p=0.0099). Decrease in ADC values was, however, not present in segments cranial and caudal to epicentres (p=0.4546 cranial and p=0.1153 caudal to epicentre; Fig. 4). Interestingly, no significant differences of ADC values were found between paraplegic dogs with a chronic state of SCI and control values at any localization (p=0.1436 epicentres, p=0.1348 cranially and p=0.1794 caudally). Neither body weight (p=0.3202 epicentres,
p=0.1266 cranially and p=0.2885 caudally) nor age (p=0.1818 epicentres, p=0.5059 cranially and p=0.8419) had an influence on ADC values among the different groups at each evaluated spinal cord segment.

Fig. 4: Tukey boxplots depicting distribution of apparent diffusion coefficient (ADC) values obtained from paraplegic dogs after acute or chronic spinal cord injury (SCI) at each evaluated segment. Second quartile, median, and third quartile of ADC obtained from control dogs are depicted in the background as a grey area. Significance levels between acute and chronic SCI affected dogs are shown with stars (*) and significance level between acute or chronic SCI affected dogs and controls are depicted with numerical signs (#).
3.4 Lesion extension ratio

T2W-LER in the acute SCI group displayed a mean value of 3.89 (SEM ± 0.3493; median 3.26), whereas the chronic group showed a mean value of 5.96 (SEM ± 0.7918; median 5.60). Dogs with chronic SCI had significantly higher T2W-LER than those acutely affected (p=0.0077).

Moreover, results of Pearson’s correlation tests revealed a moderate negative correlation between T2W-LER and FA values obtained from patients with acute and chronic SCI at the level of epicentres and one spinal cord segment cranially and a weak positive correlation between T2W-LER and ADC at the same localizations.

Correlations could not be found between both, FA and ADC, and T2W-LER in paraplegic dogs with acute SCI (table 3). However, a strong negative correlation and a moderate negative correlation was found between T2W-LER and FA values at the level of one spinal cord segment cranially to epicentres and at epicentres, respectively. ADC values and T2W-LER did not correlate in the chronic stage of SCI (table 3).
Table 2. Pearson’s correlation coefficients ($r$) between DTI and T2W-LER values.

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<th>Localization</th>
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<th>$P$</th>
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</table>

Abbreviations: DTI, diffusion tensor imaging; T2W-LER, T2-weighted – lesion extension ratio; FA, fractional anisotropy; ADC, apparent diffusion coefficient.
4. Discussion

In the present study, DTI values measured from the spinal cord of a relatively homogeneous population of paraplegic dogs with acute or chronic SCI were characterized and compared.

In dogs with chronic SCI, FA values were lower than in both, controls and dogs with an acute SCI at all spinal cord localizations evaluated. Chronic state of SCI is the result of complex adaptation responses engaging vascular changes, free radical formation, ionic imbalances, inflammation, demyelination, and apoptosis (Hagg and Oudega 2006; Mietto et al. 2015; Silva et al. 2014). Such mechanisms facilitate gliosis, activation of astrocytes and subsequent formation of intraparenchymal fluid filled cavitations through glial scar consolidation together with partial remyelination and large spaces between axons (Hu et al. 2010; Levine et al. 2011; Smith and Jeffery 2006). Consequently, massive loss of white matter tracts may cause decreases in anisotropy, which are reflected by a decrease of FA values. Low anisotropy is the hallmark of chronic SCI in humans and rodents as well (Cohen-Adad et al. 2011; Jirjis et al. 2013; Koskinen et al. 2013; Petersen et al. 2012; Wang et al. 2014). Furthermore, secondary wave injury mediated lesions extending cranially and caudally from lesion epicentre lead in some cases to myelomalacia, fluid cavity formation, and Wallerian degeneration (Bock et al. 2013; Henke et al. 2016; Kamble et al. 2011; Mietto et al. 2015). Lower perilesional FA values in paraplegic dogs affected by chronic SCI are in agreement with and supporting former observations in distant spinal cord segments of humans suffering chronic SCI (Kamble et al. 2011).

Dogs affected by acute SCI showed increased FA values at the lesion epicentre compared to controls. This finding is opposed to reported FA values of rodents after contusion, hemitrancsection or transection of the spinal cord (Li et al. 2015; Patel et al. 2016; Wang et al. 2014; Zhao et al. 2016). In contrast to the rodent model canine SCI combines contusion and
permanently compressed forces exerted over the spinal cord (Jeffery et al. 2013; Levine et al. 2011). Presence of extruded disc material in the vertebral canal at the level of epicentres may cause a reduction of space between intact or swollen axonal tracts increasing its anisotropy. Increased FA values are commonly reported in acute traumatic brain injury in humans and cytotoxic oedema in white matter tracts has been postulated as a possible cause (Eierud et al. 2014; Henry et al. 2011).

ADC revealed a wider distribution than FA in dogs with acute and chronic SCI. In a study describing healthy canine spinal cords, ADC values were less accurate than FA (Griffin et al. 2013). In the current study ADC values obtained at epicentres and perilesional differed significantly between the acute and chronic states of SCI. However, these values did not reach significance levels when compared to control dogs, with the exception of ADC metrics measured at epicentres during acute SCI. Spheroid formation as well as intra-axonal mitochondrial accumulations and permanent mechanical deformation by extruded disc material during the acute state of injury may explain the impaired diffusivity found at epicentre levels (Bock et al. 2013; Smith and Jeffery 2006). A clear differentiation of the compressed spinal cord’s white and grey matter is challenging, even when evaluating conventional T2W sequences. Therefore, no attempt was made to distinguish individual funiculi and signal deriving from whole parenchyma was considered for ROI placement as previously reported (Griffin et al. 2013; Hobert et al. 2013; Mulcahey et al. 2012).

In conventional T2W sequences, SCI can be evidenced by presence of compression and/or intramedullary hyperintense signal (Becerra et al. 1995; Bodley 2002; Lammertse et al. 2007; Yamashita et al. 1990). Intramedullary T2W hyperintensities have been associated with oedema, haemorrhage, malacia, necrosis, liquefaction and fluid filled cavitations (Byrnes et al. 2010; Hu et al. 2010; Mihai et al. 2008). As expected, dogs with acute SCI showed a lower T2W-LER than dogs from the chronic group, since progression of the secondary wave injury
may induce microstructural and MRI signal intensity changes as evidenced in perilesional and distant segments (Bock et al. 2013; Gwak et al. 2012; Kashani et al. 2010). Interestingly, lower values of FA obtained from lesion epicentres and one spinal cord segment cranially were correlated with longer T2W-LER in the chronic group, suggesting that Wallerian degeneration and enlarged space between axonal tracts and glial scar most commonly occur caudal to the lesion epicentre, and as the lesion extends, cranial segments are involved. Similar, in humans, extension of retrograde Wallerian degeneration during chronic SCI has been evidenced in axonal tracts of the dorsal column cranial to the epicentre (Becerra et al. 1995; Guleria et al. 2008; Kashani et al. 2010; Valencia and Castillo 2006).

Diffusion tensor MR was able to determine microstructural differences between acute and chronic stages of SCI, particularly regarding parenchymal anisotropy depicted by the FA value. Furthermore, FA values correlated with T2W-LER in chronic SCI. These findings suggest that measurements of FA are a promising complementary monitoring tool for microstructural evaluation of the spinal cord in dogs with chronic SCI for novel treatment implementation and emphasize the role of canine SCI as a large animal translational model for human SCI.

5. Acknowledgements

We thank the “Gesellschaft der Freunde der Tierärztlichen Hochschule Hannover” and the “Akademie für Tiergesundheit” for financial support given to the first author. The present project was partly supported by the German Research Foundation (FOR 1103, project TI 309/4-2).
Supplemental Table 1. Median and mean values of DTI metrics.

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>ADC</th>
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<tr>
<td></td>
<td>Median</td>
<td>Mean ± SEM</td>
<td>Median</td>
</tr>
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<td></td>
</tr>
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<td>Cranial</td>
<td>0.754</td>
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<td>0.885</td>
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<td>Epicentre</td>
<td>0.784</td>
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<tr>
<td>Caudal</td>
<td>0.718</td>
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<tr>
<td><strong>Chronic SCI</strong></td>
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<tr>
<td>Cranial</td>
<td>0.394</td>
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</tr>
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<td><strong>Controls</strong></td>
<td>0.693</td>
<td>0.652 ± 0.0342</td>
<td>1.344</td>
</tr>
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</table>

Abbreviations: DTI, diffusion tensor imaging; FA, fractional anisotropy; ADC, apparent diffusion coefficient; SEM, standard error of means.
Chapter 4: Evaluation of acute and chronic SCI using DTI

6. References


Chapter 4: Evaluation of acute and chronic SCI using DTI


Chapter 5: Prognostic value of pre-operative DTI for short-term motor functional recovery

5. Chapter 5: Prognostic value of pre-operative DTI for short-term motor functional recovery

A. Wang-Leandro: performed acquisition of images, generated and analysed the data, and wrote the manuscript.

J. S. Siedenburg: assisted with data analysis of T2W-LER.

M. K. Hobert: acquisition of images, advice concerning methods.

P. Dziallas: performed acquisition of images.

K. Rohn: performed statistical analysis.

V. M. Stein: was involved in the concept and design of the study and critically revised the manuscript, performed partly surgical approaches.

A. Tipold: was involved in designing of the study, discussing analysis of the data and critically revised the manuscript.

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Chapter 5: Prognostic value of pre-operative DTI for short-term motor functional recovery

Comparison of Pre-Operative Quantitative MRI and Clinical Assessment of Deep Pain Perception as Prognostic Tools for Early Recovery of Motor Function in Paraplegic Dogs with Intervertebral Disc Herniations

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Running head: Early motor function recovery after IVDH

Key words: Spinal Cord Injury, Canine, Diffusion Tensor Imaging, Paraplegia
Chapter 5: Prognostic value of pre-operative DTI for short-term motor functional recovery

Abbreviations:

AUC  area under the curve
DPP  deep pain perception
FA   fractional anisotropy
IVDH intervertebral disc herniation
MRI  magnetic resonance imaging
ROC  receiver-operating characteristics
ROI  region of interest
SCI  spinal cord injury
SD   standard deviation
T2W  T2-weighted
T2W-LER  T2-weighted - lesion extension ratio

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The present study was done at the Department of Small Animal Medicine and Surgery of the University of Veterinary Medicine Hannover, Foundation.
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Presentations at meetings:

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Conflicts of interest:

Authors disclose no conflict of interest.

Off-label antimicrobial use:

Authors declare no off-label use of antimicrobials.
Abstract

**Background:** Prognostic tools to predict early post-operative motor function recovery (MFR) after thoracolumbar intervertebral disc herniation (IVDH) in paraplegic dogs represent an opportunity to timely implement novel therapies that could shorten recovery times and diminish permanent neurological dysfunctions.

**Hypothesis:** Fractional anisotropy (FA) values obtained using diffusion tensor imaging have a higher prognostic value than a lesion extension ratio in T2-weighted images (T2W-LER) and clinical assessment of deep pain perception (DPP) for MFR.

**Animals:** 35 paraplegic dogs with diagnosis of acute or subacute thoracolumbar IVDH.

**Methods:** Prospective, descriptive observational study. At admission, absence or presence of DPP, T2W-LER and FA values were evaluated. MFR was assessed within 4 weeks after decompressive surgery. Values of T2W-LER and FA of dogs with and without MFR were compared using t-tests. Receiver-operating characteristics curve (ROC) analyses were calculated to assess validity of FA and T2W-LER measurements and Youden indices were applied for significances found in ROC analysis. Sensitivity and specificity of DPP was calculated as a dichotomous model using Fisher’s exact test.

**Results:** No differences were found between groups regarding T2W-LER. FA values differed statistically when measured caudally of lesion epicenter being higher in dogs without MFR compared to dogs with MFR (p=0.023). ROC analysis revealed significance in FA values measured caudally of the lesion epicenter (p = 0.033, area under the curve = 0.715). Using a cut-off value of FA=0.660, the technique showed a sensitivity of 80% and a specificity of 55%. Evaluation of DPP displayed a significance of p=0.007, sensitivity of 75 % and specificity of 73.3 %.
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Conclusions and clinical importance: Evaluation of DPP showed a similar sensitivity and a better specificity predicting early MFR than quantitative MRI.

1. Introduction

Acute thoracolumbar intervertebral disc herniation (IVDH) is a common neurological disease in dogs that may lead to permanent sensorimotor and visceral function impairments (Aikawa et al. 2012; Fluehmann et al. 2006; Olby et al. 2003). Thoracolumbar IVDH occurs predominantly in chondrodystrophic dogs due to early degeneration of intervertebral discs and exerts a mixture of contusive and compressive forces to the spinal cord (Hansen 1952; Jeffery et al. 2013; Smolders et al. 2013).

Current treatment for paraplegic dogs with IVDH is addressed to eliminate the source of primary mechanical damage and consists of surgical decompression of the spinal cord (Brisson 2010; Kube and Olby 2008; McKee 1992; Scott 1997). However, shortly after the primary injury, a complex and dynamic cascade of cellular processes including inflammation, edema, ischemia, reactive species liberation, excitotoxicity, microglial and astrocytic activation occur (Bock et al. 2013; Kwon et al. 2004; Silva et al. 2014; Spitzbarth et al. 2011). This spectrum of responses is known as the “secondary injury” and it occurs seconds to weeks after the primary injury (Hagg and Oudega 2006; Silva et al. 2014).

Research on novel therapies is performed and aims to neutralize or diminish the effects of the early secondary wave damage (Granger et al. 2014; Raspa et al. 2016). Early motor function recovery (MFR) has been rarely explored as an outcome measurement, but represents an opportunity to timely implement novel therapies that could shorten recovery times and contribute to diminish permanent neurological dysfunctions.
Assessment of deep pain perception (DPP) during neurological examination, composition of cell populations and biomarkers present in the cerebrospinal fluid (CSF) and quantitative magnetic resonance imaging (MRI) have been formerly evaluated as prognostic factors for long-term functional recovery in dogs with thoracolumbar IVDH (Aikawa et al. 2012; Boekhoff et al. 2012; Ito et al. 2005; Jeffery et al. 2016; Levine et al. 2014; Levine et al. 2009; Olby et al. 2003; Penning et al. 2006; Roerig et al. 2013; Witsberger et al. 2012). Evaluation of DPP and length of intramedullary hyperintense signal in sagittal T2-weighted MRI were proven to be useful predictive tools for long-term MFR (Aikawa et al. 2012; Boekhoff et al. 2012; Ito et al. 2005; Levine et al. 2009).

Diffusion tensor imaging (DTI) is a state-of-the-art modality of MRI that allows in vivo microstructural evaluation of white matter tracts by quantifying water molecule diffusion (Beaulieu 2002). DTI of the spinal cord has been increasingly applied for numerous diseases including SCI in different animal models and humans (Li et al. 2015; Martin et al. 2016; Sasiadek et al. 2012; Vedantam et al. 2014). Fractional anisotropy (FA) is a unitless value that ranges from 0 to 1. FA equal zero represents unrestricted directional diffusion of water molecules and FA equal one represents a completely restricted diffusion in only one possible direction (Beaulieu 2002; Hendrix et al. 2015). Therefore, highly organized tissues such as white matter tracts provide a homogeneous anisotropic environment for water molecule diffusion (Lerner et al. 2014; Vedantam et al. 2014). Recently, feasibility of DTI of the canine healthy spinal cord has been reported and the tissue was characterized (Griffin et al. 2013; Hobert et al. 2013; Yoon et al. 2016). Since a correlation between parenchymal damage of the spinal cord and severity of neurological deficits was found by Henke and colleagues (2013), the introduction of DTI as an objective clinical tool for assessment of structural integrity of the spinal cord may be valuable for pre-operative determination of prognosis.
Chapter 5: Prognostic value of pre-operative DTI for short-term motor functional recovery

Therefore, the aim of this study is to evaluate the potential pre-operative prognostic value for early MFR in a population of dogs with thoracolumbar IVDH using three techniques: measurement of the extension of spinal cord compression and hyperintensity in sagittal T2W sequences at the level of SCI, FA values obtained from DTI sequences, and clinical assessment of DPP. The hypothesis should be proven, that DTI parameters will show a higher sensitivity and specificity than a lesion extension ratio in T2W images (T2W-LER) and assessment of DPP predicting post-operative MFR.

2. Materials and Methods

2.1 Animals

For the present study, dogs admitted to the Department of Small Animal Medicine and Surgery of the University of Veterinary Medicine Hannover between June 2013 and April 2015 were prospectively recruited. The dogs had to fulfil the following inclusion criteria: acute paraplegia (0-7 days since observed onset of clinical signs) or subacute paraplegia (8-28 days since onset of clinical signs; Griffin et al. 2015; Hu et al. 2010; Levine et al. 2009), SCI confined to the T3-L3 spinal cord segments and a body weight less than 20 kg. Time elapsed between non-ambulatory state of the dog and admission to the clinic was used for classification of acute and subacute paraplegia (Jeffery et al. 2016). At admission, each dog underwent a physical and neurological evaluation, plain radiographic imaging of the thoracic and lumbar vertebral column and MRI of the thoracolumbar spinal cord to diagnose IVDH. Furthermore, complete blood workup, serum biochemistry and CSF analysis were performed in order to exclude differential diagnoses. IVDH was confirmed during surgery, all dogs were treated with decompressive surgery of the spinal cord and appearance of motor function recovery was documented within 4 weeks thereafter. Dogs were excluded from the study, if a
compression caudal to the L4 vertebral body or neurologic deficits compatible with a lower motor neuron lesion were present. Post-operative MFR was noted, when dogs regained voluntary movement of the hind limbs together with presence of DPP within 4 weeks after decompressive surgery. This study was performed after the approval of the German Animal Welfare instances (Number: 33.9-42502-04-11/0661) and the written owners’ consent for each examination.

### 2.2 Assessment of deep pain perception

Dogs were tested for presence or absence of DPP during clinical evaluation. Presence of DPP was defined as an obvious and reproducible behavioral response that could be interpreted as pain towards a noxious stimulus (i.e. whining, sudden turning the head and/or biting attempts towards the source of stimulus). For the test, digits of both hind limbs were clamped using forceps (Aikawa et al. 2012; Jeffery et al. 2016; Ruddle et al. 2006).

### 2.3 Magnetic resonance imaging

MRI scans were performed under general anesthesia using a 3 tesla scanner (Philips Achieva, Phillips Medical Systems, Eindhoven, The Netherlands) and protocols consisted of sagittal and transversal T2W and transversal DTI sequences as previously reported (Griffin et al. 2013; Hobert et al. 2013). T2W sequences were assessed by board certified neurologists (AT and/or VS) in order to determine localization of SCI for subsequent surgical procedures. Lesion extension ratio in T2W images (T2W-LER) was defined as lengths of spinal cord compression and intramedullary hyperintense signal expressed as a ratio in relation to length of vertebral body of L2 (Boekhoff et al. 2012). T2W-LER was evaluated in sagittal planes using the software easyVET® (Version 8.0.0.03/R3, 2015, Isernhagen, Germany).
Moreover, using the software Extended MR workspace® (Version 2.6.3.4, 2012, Philips Medical Systems, the Netherlands), T2W images were used as templates for placement of regions of interest (ROIs) in transversal DTI sequences. ROIs were placed in signals deriving from the spinal cord in FA maps directly dorsally of intervertebral disc spaces at the epicenter of the lesion and one vertebral body cranial and caudal to the epicenter. Epicenters were defined as spinal cord segments with compression evidenced in T2W sequences. ROIs were placed using individual voxels in order to avoid measuring diffusion metrics deriving from CSF or epidural fat. Afterwards, voxels were fused and values of FA were obtained from each ROI.

2.4 Statistical Analysis

Dogs were divided into two groups: dogs with and without post-operative MFR. Age and body weight between groups were compared via t-tests. Variance analyses for FA values at each independent localization were performed. Receiver-operating characteristics curve (ROC) analysis were calculated to assess validity of FA and T2W-LER measurements and Youden indices were applied for significances found in ROC analysis. Sensitivity and specificity of DPP was calculated as a dichotomous model using Fisher’s exact test. Continuous variables were depicted descriptively as mean (± standard deviation; SD) for normally distributed variables. Significance level was considered as p<0.05. SAS® software (version 9.2, SAS Institute, Cary, NC, USA) and GraphPad Prism® (version 5, GraphPad Software, CA, USA) were used for analysis of data and graphic generation, respectively.
3. Results

3.1 Animals

Thirty-five dogs, 19 males and 16 females, fulfilled the inclusion criteria. Thirty-three dogs presented an acute and two dogs a subacute SCI due to IVDH. The mean time between onset of non-ambulatory status and pre-operative clinical examination was 2.2 days (median 1 day, range 0-22 days). Most presented dogs were Dachshunds with 17 individuals and 7 mixed-breed dogs. Furthermore, three French bulldogs, two Jack Russell Terrier, two Shih-Tzu and one dog of each of the following breeds were included: Chihuahua, small Munsterlander pointer and Lhasa Apso. Twenty dogs showed early MFR within 4 weeks after surgical decompression of the spinal cord, whereas 15 dogs did not improve. No differences in age, weight or time since onset of clinical signs were found between groups (Table 1). Most common localizations for IVDH were Th12/13 and Th13/L1 (Fig. 1).

Table 1. Comparison of age, body weight, and time between onset of non-ambulatory status and clinical examination between groups.

<table>
<thead>
<tr>
<th></th>
<th>MFR (n=19)</th>
<th>No (n=16)</th>
<th>MFR (n=19)</th>
<th>P</th>
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<tbody>
<tr>
<td>Age (years; mean ± SD)</td>
<td>5.5 ± 2.8</td>
<td>6.8 ± 3.5</td>
<td>0.225</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg; mean ± SD)</td>
<td>9.8 ± 4.2</td>
<td>9.2 ± 3.1</td>
<td>0.691</td>
<td></td>
</tr>
<tr>
<td>Time between onset of non-ambulatory status and clinical examination (days; mean ± SD)</td>
<td>2.9 ± 5.2</td>
<td>1.3 ± 2.0</td>
<td>0.275</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MFR, motor function recovery; SD, standard deviation.
3.2 T2W - lesion extension ratio

Mean T2W-LER measured from dogs without post-operative MFR was 4.46 ± 1.73 and with post-operative MFR 3.33 ± 1.96. Variance analysis revealed no significant differences between dogs with and without MFR after decompressive surgery (p = 0.085). ROC analysis displayed no significant differences for prediction of early MFR between groups (p = 0.0972, area under the curve (AUC) = 0.730; Fig. 2).
3.3 Fractional anisotropy

Mean values of FA obtained at the level of epicenters were 0.764 ± 0.067 and 0.775 ± 0.073 for dogs with post-operative MFR and without post-operative MFR, respectively. One vertebral body cranially, mean FA value from dogs with MFR was 0.714 ± 0.104, whereas in dogs without MFR values of 0.741 ± 0.093 were determined. Furthermore, measurements of
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FA one vertebral body caudally to epicenters had a mean of 0.658 ± 0.093 for dogs with MFR and 0.735 ± 0.094 for dogs without MFR. Variance analysis showed no significant differences between groups at lesion epicenters (p=0.947) and one vertebral body cranial to the epicenter (p=0.442); however, significant differences in FA were evidenced in the spinal cord one vertebral body caudal to the epicenters (p=0.023).

Similarly, ROC analysis of FA values to predict post-operative MFR revealed no significant differences between groups at the level of epicenters (p=0.633, AUC=0.568) and one vertebral body cranially (p=0.430, AUC=0.568). Nonetheless, a significant difference was found caudal to the epicenter (p= 0.033, AUC=0.715; Fig. 2). Youden index calculations applied to FA values caudal to the lesion epicenter revealed a sensitivity of 80 % and a specificity of 55 % for prediction of negative outcome using a cut-off value of FA > 0.660.

3.4 Deep pain perception

Evaluation of DPP before decompressive surgery revealed a positive response in 19 dogs and a negative response in 16 dogs. Within the population of dogs with post-operative MFR, 25 % (5/20) had a negative DPP response at admission; whereas in dogs without MFR, DPP could not be elicited in 73.3 % (11/15) of the dogs before decompressive surgery. Fisher’s exact test for evaluation of DPP as a prognostic tool for early functional recovery displayed a significance of p=0.007, sensitivity of 75 % and specificity of 73.3 %.
4. Discussion

This study prospectively evaluates pre-operative measurements of spinal cord lesion extension in conventional T2W MRI sequences, DTI parameters and clinical assessment of DPP as prognostic factors for early MFR in a population of paraplegic dogs with acute and subacute SCI. Dogs were tested for presence or absence of DPP, length of SCI was measured in sagittal T2W sequences and values of FA were obtained from epicenter of the lesion and one vertebral body cranially and caudally. After decompressive surgery, neurological examinations were repeated and data from dogs with and without post-operative MFR within 4 weeks were compared.

Evaluation of prognostic tools for early MFR in paraplegic dogs with SCI have been uncommonly reported (Chamisha et al. 2015; Roerig et al. 2013). Establishment of clinical tools that could provide a prognostic value in the time window of early MFR may have an impact on timely selection of patients with unfavorable prognosis for early implementation of novel therapies.

In the population of affected dogs, Dachshund was the breed presented the most and Th12/13 and Th13/L1 occurred most frequently as localization of thoracolumbar IVDH, in ten and eleven cases respectively, as previously reported (Brisson 2010; Levine et al. 2009; Olby et al. 2004; Tanaka et al. 2004). Chondrodystrophic breeds such as Dachshunds are frequently affected by early degeneration of intervertebral discs and presence of intercapital ligaments may partially prevent intervertebral discs to herniate in cranial segments of the thoracic vertebral column (Aikawa et al. 2012; Brisson et al. 2004; Hansen 1952; Levine et al. 2009; Olby et al. 2004; Olby et al. 2003; Priester 1976; Smolders et al. 2013).

Pre-operative evaluation of DPP revealed that 78.9 % of dogs with intact DPP (15/19) and 31.3 % of dogs with absent DPP (5/16) developed post-operative MFR within 4 weeks after
decompressive surgery. Assessment of DPP remains an accepted and commonly applied test for prognosis of recovery in paraplegic dogs with IVDH (Jeffery et al. 2016), although its performance and interpretation has been considered as controversial (Speciale 2003; Thomovsky and Chen-Allen 2013). For long-term functional recovery, presence of DPP in non-ambulatory dogs with thoracolumbar IVDH is associated with positive outcomes in nearly 100 % of the cases; however, absence of DPP has been correlated with a recovery rate of approximately 50 % (Aikawa et al. 2012; Davis and Brown 2002; Jeffery et al. 2016; Jeffery et al. 2013; Ruddle et al. 2006). A clear difference is detected in the current study with lower accuracy of DPP to predict early MFR in comparison to formerly reported prediction of long-term MFR. Late-onset recovery of ambulation in paraplegic dogs with IVDH after surgical decompression can appear up to 6 months thereafter and ranges from 13.4 % to 31.8 % of which some dogs regain ambulation without regaining DPP (Aikawa et al. 2012). However, for early application of novel treatment strategies in dogs which would fail standard therapy, prediction of early MFR becomes useful and necessary allowing selection of target populations.

Values of T2W-LER displayed no significant differences between dogs with and without early MFR. This finding contrasts previous studies, where longer intramedullary hyperintensities in sagittal T2W sequences were predictive for unfavorable long-term outcome using 0.3 and 1 tesla magnetic fields (Boekhoff et al. 2012; Ito et al. 2005; Levine et al. 2009). Presence of intramedullary T2W hyperintensities during acute and subacute stages of SCI is assumed to be a consequence of edema, hemorrhage and necrosis (Jeffery et al. 2013; Kulkarni et al. 1987). This study intended not only to quantify hyperintense signal in sagittal T2W sequences but the complete extension of the SCI, including length of intramedullary intensity changes as well as extramedullary spinal cord compressions. However, pre-operative T2W-LERs seem not to be of prognostic value for early MFR.
To the author’s knowledge, this study is the first report to evaluate DTI parameters as prognostic tool for MFR in paraplegic dogs with IVDH. Increased pre-operative FA values were found one vertebral body caudal to the lesion epicenter in dogs without MFR compared to dogs that showed MFR suggesting the occurrence of cytotoxic edema and axonal swelling (Facon et al. 2005; Henry et al. 2011; Wilde et al. 2008). Although a difference was found, the ability of DTI parameters to predict early MFR was lower than evaluating DPP pre-operatively, displaying a similar sensitivity but a remarkably lower specificity. Therefore, the assessment of pre-operative DTI parameters did not offer benefits over DPP assessment.

In conclusion, ability to predict early post-operative MFR was evaluated for clinical assessment of DPP, sagittal T2W sequences and DTI parameters of the spinal cord of paraplegic dogs with acute and subacute IVDH. The hypothesis could not be proven, that DTI shows a higher sensitivity and specificity than a lesion extension ratio in T2W images (T2W-LER) and assessment of DPP predicting post-operative MFR. In fact, presence of intact DPP had a similar sensitivity and a better specificity in predicting early functional recovery than quantitative MRI, herewith still emphasizing the importance of clinical examination.
References


Chapter 5: Prognostic value of pre-operative DTI for short-term motor functional recovery


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**Chapter 5: Prognostic value of pre-operative DTI for short-term motor functional recovery**


Chapter 5: Prognostic value of pre-operative DTI for short-term motor functional recovery


6. Chapter 6: Discussion

In the present study, DTI was evaluated as a tool for temporal characterization and prognostic determination in paraplegic dogs with thoracolumbar SCI. For this purpose, values of FA and ADC were obtained from the spinal cord of dogs with acute or chronic SCI at lesion epicentre and one vertebral body cranially and caudally. Moreover, a population of dogs was scanned three months after showing motor function recovery (MFR) following surgical decompression of the spinal cord.

The aims of this study were: (1) to prove feasibility of DTI to detect diffusion changes in the acute or chronic injured spinal cord of paraplegic dogs compared to values from control dogs, (2) to describe temporal evolvement of DTI metrics in paraplegic dogs with recovery of motor function after decompressive surgery, (3) to compare values of DTI between acute and chronic stages of SCI, and (4) to determine the pre-operative prognostic value of DTI in paraplegic dogs with thoracolumbar SCI.

Traumatic SCI is a devastating neurological disease of the central nervous system that can lead to permanent loss of sensorimotor function and causes severe economic and social burdens (Jazayeri et al. 2015; Krueger et al. 2013; Silva et al. 2014). Research on diagnostic, prognostic, and novel treatment strategies for human traumatic SCI has been traditionally performed in the rodent model (Kelley et al. 2014; Kim et al. 2010; Loy et al. 2005; Mihai et al. 2008). Nonetheless, this model presents major discrepancies with human SCI, such as absence of lesion inhomogeneity, lack of environmental influence and/or concomitant medical conditions and small size of individuals (Jeffery et al. 2011). The dog is a well-established large animal translational model for SCI and represents a unique opportunity to bridge the gap between rodents and humans (Jeffery et al. 2011; Jeffery et al. 2006; Levine et
The results of the present study emphasize pathophysiological differences between induced and naturally occurring SCI in rodents and dogs, respectively, and highlight the importance of dogs as a large animal translational model for human traumatic SCI to implement novel treatment strategies.

6.1. DTI metrics during acute SCI

Description of the healthy spinal cord in dogs using DTI was reported by Hobert et al. (2013), Griffin et al. (2013) and most recently by Yoon and collaborators (2016). We evaluated dogs with acute onset of paraplegia (≤ 7 days) caused by IVDH or exogenous trauma using DTI and compared diffusion metrics with control values (Wang-Leandro et al. 2016).

In paraplegic dogs with acute contusive-compressive SCI, FA values were increased compared to control dogs, indicating that an acute reduction of vertebral canal diameter and consequently compression of white matter tracts may have an important effect in directionality of diffusion, making it more anisotropic at this time point (Wang-Leandro et al. 2016; Fig. 5).

Decreased values of ADC in the compressed spinal cord were consistently found and associated with the acute stage of SCI in dogs (Wang-Leandro et al. 2016). Presence of disc material within the vertebral canal and mitochondria accumulation in swollen and normal appearing axons may represent a physical obstacle for optimal water molecule diffusion (Bock et al. 2013; Smith and Jeffery 2006).

Characterization of acute SCI using DTI in humans has been rarely reported and show differences concerning methods, neurological status of patients evaluated and protocols used
(Cheran et al. 2011; Facon et al. 2005; Shanmuganathan et al. 2008; Vedantam et al. 2013). The limited amount of information may be due to the fact that patients with acute SCI frequently need to be surgically treated to decompress the spinal cord or stabilize the vertebral column, therefore favouring short time MRI scans (Wang-Leandro et al. 2016). The rodent model has been therefore widely used to describe diffusion metrics in acute SCI, inducing contusion, hemi-transection, total transection or distraction of the spinal cord after performing a dorsal laminectomy in anesthetized individuals (Jirjis et al. 2013; Kelley et al. 2014; Kim et al. 2010; Li et al. 2015; Patel et al. 2016; Wang et al. 2014).

A drastic reduction of FA values is reported as a common finding in rodent SCI and increase or decrease of ADC can be found directly after acute SCI in this species (Jirjis et al. 2013; Li et al. 2015; Patel et al. 2016; Wang et al. 2014). Although the rodent model provide a highly homogeneous laboratory environment, it has the limitation of ignoring the compressive component commonly found in human acute traumatic SCI (Levine et al. 2011). The presence of extramedullary compression may have an effect on reducing extracellular space and therefore increasing anisotropy in dogs and humans with SCI (Facon et al. 2005; Wang-Leandro et al. 2016).
Chapter 6: Discussion

A

$\lambda_1 > \lambda_2, \lambda_3$

B

$\lambda_1 >>> \lambda_2, \lambda_3$

C

$\lambda_1 \approx \lambda_2 \approx \lambda_3$
Fig. 5. Diffusion ellipsoids in the healthy and injured canine spinal cord. Schematic representation of diffusion ellipsoids in a sagittal view of a (A) healthy uninjured spinal cord, (B) acute contusive compressive spinal cord injury (SCI) caused by an intervertebral disc herniation, and (C) chronic SCI with an intramedullary cavity. Cranial and caudal are considered on the left and right side of the figure, respectively. Anisotropy is increased during acute contusive-compressive SCI (B) and decreased during chronic SCI (C) compared with the healthy spinal cord (A).

6.2. DTI metrics during chronic SCI

Chronic stage of SCI presents different histological and pathophysiological characteristics than the acute stage (Anwar et al. 2016; Silva et al. 2014; Smith and Jeffery 2006). To address these differences in parenchymal structure, a population of fifteen dogs with chronic SCI (> 28 days) was evaluated using DTI and conventional T2W sequences; values of T2W-LER, FA and ADC were compared between paraplegic dogs with acute or chronic SCI and control dogs.
Values of FA obtained from the spinal cord of dogs with chronic SCI were significantly lower than FA values from dogs with acute SCI and control values; ADC values of the chronic stage of SCI showed no differences compared to control values and a correlation was found between T2W-ELR and FA values obtained from lesion epicentre and one vertebral body cranially.

Decreased FA values of the spinal cord are considered the hallmark in humans of chronic SCI (Martin et al. 2016). Results displayed in this study are in agreement with human studies describing a less anisotropic diffusion of water molecules in the spinal cord in the chronic stage of SCI. Massive parenchymal destruction, myelino- and axonophagia, Wallerian degeneration and formation of glial scar tissue and fluid filled cavitations cause an increased space between axons and therefore a less homogeneous microstructural environment (Hu et al. 2010; Levine et al. 2011; Smith and Jeffery 2006; Fig. 5).

Values of ADC obtained from the spinal cord in the chronic stage of SCI are reported to be inconsistent in human literature (Martin et al. 2016). Measurements of ADC obtained from the healthy canine spinal cord reported by Hobert et al. (2013) and Griffin et al. (2013) confirmed a wider range of values and less accuracy in comparison to FA values. Nonetheless, ADC values from the spinal cord of dogs with chronic SCI displayed no significant differences with controls in this study as expected. Tissues without mechanical restriction to diffusion should have about the same diffusion magnitude level.

Interestingly, lower values of FA obtained from lesion epicentres and one vertebral body cranially were correlated with longer T2W-ELR in the chronic group, suggesting that Wallerian degeneration and enlarged space between axonal tracts and glial scar most commonly occur caudal to the lesion epicentre, and as the lesion extends, cranial segments are involved. Similar, in humans, extension of retrograde Wallerian degeneration during chronic
SCI has been evidenced in axonal tracts of the dorsal column cranial to the epicentre (Becerra et al. 1995; Guleria et al. 2008; Kashani et al. 2010; Valencia and Castillo 2006).

Most information available from the rodent model relies on follow up studies after acute SCI in populations showing some degree of motor function recovery. Such an approach excludes a population of animals with unfavourable prognosis (Kelley et al. 2014; Wang et al. 2014). Hu and collaborators (2010) reported the consolidation of glial scars to take place four weeks after initial injury in rats and dogs. Paraplegic dogs with a chronic SCI evaluated in this study were scanned more than 28 days after showing a non-ambulatory state. After this time point the chronic stage of injury is most probably reached. As such these dogs are representing a suitable population for clinical trials evaluating new therapeutic approaches (Granger et al. 2012; McMahan et al. 2015; Sarmento et al. 2014).

6.3. DTI as monitoring technique for functional recovery

Versatility of DTI allows in vivo evaluation of the spinal cord at different time points after initial SCI (Li et al. 2015; Wang et al. 2014). Temporal evolvement of DTI values was assessed in a population of dogs with acute or subacute SCI with motor function recovery subsequent to surgical decompression of the spinal cord; at follow up examinations, three months after MFR, all dogs were able to walk, one with support (Wang-Leandro et al. 2016).

Values of FA and ADC showed no significant differences compared to control values at follow up examinations suggesting an association between clinical and microstructural findings (Wang-Leandro et al. 2016). Additionally, the current study confirms the presumed anisotropy alterations produced by compressive forces and may indicate that early decompression of the spinal cord may have led to effective reperfusion (Fehlings and Perrin 2005); intrinsic axonal regeneration and remyelination mechanisms taking place in the
recovery phase could have contributed to the parenchymal integrity observed in follow up scans (Bock et al. 2013).

Differences of DTI metrics between acute and chronic stages of SCI and association with motor function recovery open the possibility to use this technique as a monitoring tool during implementation of novel treatment strategies. Transplantation of stem cells or differentiated cell types with regenerative capacities is a current focus of research in clinical trials of animals with SCI (Granger et al. 2014; Hoffman and Dow 2016). Electrophysiological tests are commonly used for functional assessment during recovery phase (Granger et al. 2012; Jeffery et al. 2011; McMahill et al. 2015; Sarmento et al. 2014; Tamura et al. 2015); however, DTI could represent a useful complementary technique for structural evaluation.

6.4. Prognostic utility of DTI

In the third part of the present study DTI was evaluated as a prognostic tool before decompressive surgery. The hypothesis should be proven that DTI metrics would represent a prognostic marker for recovery in SCI. Capability of DTI to predict a negative outcome was compared with clinical assessment of DPP and extension of intramedullary lesions evaluated in sagittal T2W MRI sequences in dogs with acute SCI.

Interestingly, differences in FA values were detected one vertebral body caudally to the lesion epicentre between dogs with and without motor function recovery. Populations of dogs showing no MFR displayed higher FA values, suggesting an extended and increased amount of cytotoxic oedema and axonal swelling (Facon et al. 2005; Henry et al. 2011; Wang-Leandro et al. 2016; Wilde et al. 2008). Although DTI values were able to differentiate between dogs with and without MFR, its ability to predict a negative outcome was lower than DPP evaluation performed during neurological examination. This finding contrasts with
studies performed by Kim and colleagues (2010) and Patel and collaborators (2016) in rodents, where diffusion metrics could accurately predict long-term functional recovery. However, paraplegic dogs with acute IVDH present a wide variety of compression degree, contusion, extension of lesion and haemorrhage before decompressive surgery (Bock et al. 2013; Henke et al. 2013; Penning et al. 2006).

Severe acute SCI includes complex and heterogeneous functional and structural changes that oversimplified clinical classifications seem to overlook; therefore, quantitative methods that permit reliable evaluation of residual tissue spared and not damaged by SCI are urgently needed (Krishna et al. 2014). Development of objective and sensitive techniques that could be used during early phases of SCI for detection of reversible changes of the spinal cord tissue may have an impact in patient selection for novel and/or multimodal therapy application in patients with unfavourable prognosis after clinical examination (Krishna et al. 2014). As a useful translational model, canine severe SCI is an example for challenging decision making scenarios. Clinicians are frequently asked by dog owners to provide prognosis regarding functional recovery (Jeffery et al. 2016; Jeffery et al. 2013). Although sensitivity of DTI for early selection of patients for novel therapy implementations is not very high before decompressive surgery, exploring different time windows in the first post-operative weeks could elucidate its ability to determine prognosis (Fig. 6).
Fig. 6. Possible time window for patient selection. Schematic representation of fractional anisotropy (FA) values at each evaluated time point. Increased FA was common in dogs with acute spinal cord injury (SCI). FA values from dogs scanned three months after showing motor functional recovery (MFR) showed no differences in comparison to control values and decreased FA values were found in dogs with chronic SCI compared to control values. Time window for potential selection of patients for novel therapy implementation using diffusion tensor imaging (DTI) is signalized with red.

As conclusions, temporal evolvement and characterization of SCI using DTI was successfully evaluated in a relatively homogeneous population of dogs with acute or chronic SCI. With development and implementation of novel treatment strategies for SCI, DTI may represent a practical non-invasive method for therapeutic effect monitoring. Clinical techniques that allow objective assessment and characterization of the effects of the secondary wave injury and its evolvement into chronic injuries may clarify the ideal time for selection and treatment implementation.
7. Chapter 7: Summary

Temporal Characterization and Prognostic Value Determination of Severe Spinal Cord Injuries in Paraplegic Dogs Using in vivo Diffusion Tensor Imaging

Adriano Wang Leandro

Canine spinal cord injury (SCI), a common and devastating neurological disease affecting the central nervous system, can lead to permanent sensorimotor and visceral dysfunction. SCI in dogs is frequently caused by intervertebral disc herniations (IVDH) and is characterized by contusive-compressive forces. Diffusion tensor imaging (DTI), a modality of magnetic resonance imaging (MRI), permits in vivo and non-invasive evaluation of integrity of white matter tracts by quantification of diffusion direction, expressed by fractional anisotropy (FA) values and magnitude, expressed by apparent diffusion coefficient (ADC) values. Canine SCI is a well-recognized large animal translational model for human traumatic SCI, since pathophysiology, clinical signs and clinical approach similarities between both species exist.

Therefore, the aims of these study were: (1) to prove feasibility of DTI to detect diffusion changes in the acute or chronic injured spinal cord of paraplegic dogs compared to values from control dogs, (2) to describe temporal evolvement of DTI metrics in paraplegic dogs with recovery of motor function after decompressive surgery, (3) to compare values of DTI between acute and chronic stages of SCI, and (4) to determine the pre-operative prognostic value of DTI in paraplegic dogs with thoracolumbar SCI.

In the first part of this thesis, MRI scans were performed and DTI values compared in a population of 17 dogs with acute and two dogs with subacute IVDH and 6 control dogs at two
different time points: before undergoing surgical decompression of the spinal cord and three months after showing motor function recovery (MFR). Increase of FA values were found at the epicentre of the lesion and decrease of ADC values were found at the epicentre of the lesion as well as perilesional in paraplegic dogs compared to controls, indicating a restriction in diffusion direction and magnitude during contusive-compressive SCI. Three months after functional recovery, no differences were found between groups, suggesting an association between DTI metrics and functional recovery.

In the second part, DTI values obtained from the spinal cord of 32 paraplegic dogs with acute SCI, 15 with chronic SCI and 6 control dogs were compared and correlated with measurements of lesion extension in conventional sagittal T2-weighted sequences (T2W-LER). Acute SCI was characterized by an increase of FA values and a decrease of ADC values at the level of the epicentres compared to control values. FA values from dogs with chronic SCI were decreased compared to control values, suggesting that histological features of the chronic stage leading to increased space between axons facilitate a low anisotropic environment for water molecule diffusion. Moreover, values of FA cranially to the lesion epicentre in dogs with chronic SCI correlated with longer T2W-LER, indicating that Wallerian degeneration and cavity formation takes place more commonly caudal to the lesion epicentre and as the lesion expands, cranial segments are affected as well.

In the third and last part of this thesis, FA was evaluated as a pre-operative prognostic tool for short-term functional outcome in paraplegic dogs with IVDH and compared with T2W-LER and clinical assessment of deep pain perception (DPP). Thirty-three paraplegic dogs with acute and two with subacute SCI due to IVDH were prospectively included in the study. All dogs underwent neurological examination and MRI scans with T2W and DTI sequences before surgical decompression of the spinal cord were performed. MFR was monitored post-operatively within 4 weeks. Post-operative MFR was predicted by DPP with a sensitivity of
75% and a specificity of 73.3%. T2W-LER values showed no significant differences between dogs with and without MFR, revealing no prognostic ability for short-term functional recovery. Furthermore, FA values obtained caudally to the lesion epicentre could predict post-operative MFR with 80% sensitivity and 55% specificity using a cut-off value of FA>0.660. Pre-operative DTI displayed no benefits over DPP assessment.

In conclusion, temporal evolvement and characterization of SCI using DTI was successfully evaluated in a relatively homogeneous population of dogs with acute or chronic SCI. With development and implementation of novel treatment strategies for SCI, DTI may represent a practical non-invasive method for therapeutic effect monitoring. Clinical techniques that allow objective assessment and characterization of the effects of the secondary wave injury and its evolvement into chronic injuries may identify the ideal time for patient selection and treatment implementation.
8. Chapter 8: Zusammenfassung

Temporäre Charakterisierung und Bestimmung des prognostischen Wertes von in vivo Diffusion Tensor Imaging beim Rückenmarkstrauma des Hundes

Adriano Wang Leandro

Rückenmarkstrauma bei Hunden (spinal cord injury, kurz: SCI) ist eine häufig vorkommende und schwerwiegende Erkrankung des zentralen Nervensystems, die zu dauerhaften Schäden der sensorischen, motorischen und viszeralen Fähigkeiten führen kann. SCI bei Hunden wird häufig durch Bandscheibenvorfälle (intervertebral disc herniation, kurz: IVDH), die kontusive und kompressive Kräfte auslösen, verursacht. Diffusion Tensor Imaging (Kurz: DTI) ist eine der Modalitäten der Magnetresonanz, die in vivo nicht-invasive Auswertungen über den Zustand axonaler Fasern erlaubt. Sie kann einerseits die Diffusionsrichtung quantifizieren, was durch den „fractional anisotropy“ (FA) Wert ausgedrückt wird, und andererseits das Diffusionsausmaß mit Hilfe des „apparent diffusion coefficient“ (ADC) Wertes beschreiben.

Das Rückenmarkstrauma bei Hunden ist ein anerkanntes translationales Tiermodell für humane Rückenmarkstraumata, da Pathophysiologie, klinische Symptome und klinische Abklärung bzw. Therapie bei beiden Spezies ähnlich sind.

Die Ziele dieser Studie waren: (1) Machbarkeitsstudie, ob DTI bei akuten, subakuten und chronischen Rückenmarksverletzungen von paraplegischen Hunden im Vergleich zu Kontrolltieren Diffusionsänderungen erkennen lässt, (2) die Beschreibung des zeitlichen Verlaufs der DTI-Metrik bei paraplegischen Hunden mit Wiedererlangen motorischer
Funktion nach operativer Dekompression, (3) Vergleich der DTI Werte zwischen der akuten und chronischen Phase des Rückenmarkstraumas, und (4) Bestimmung, ob DTI prä-operativ eine prognostische Aussage bei paraplegischen Hunden mit thorakolumbalen SCI hat.


Im zweiten Teil der These wurden die Werte von 32 paraplegischen Hunden mit akutem SCI, 15 mit chronischem SCI und 6 Kontrolltieren miteinander verglichen und mit den Messungen der Länge der Läsion in konventionellen sagittalen T2-gewichteten Sequenzen (T2W-LER) korreliert. Bei akuten SCI konnte eine Erhöhung der FA-Werte und eine Erniedrigung der ADC-Werte auf Höhe der Läsonsepizentren im Vergleich zu Kontrollwerten gemessen werden. Die FA-Werte von Hunden mit chronischem SCI waren im Vergleich zu den Kontrollwerten erniedrigt, was die Annahme zulässt, dass histologische Veränderungen in der chronischen Phase, die zur Erweiterung der Zwischenräume zwischen den Axonen beitragen, eine isotrope Umgebung für die Diffusion von Wassermolekülen ermöglichen. Außerdem korrelierten die FA-Werte cranial der Läsion bei Hunden mit chronischem Rückenmarkstrauma mit längeren T2W-LER. Eine Wallersche Degeneration und Entwicklung von intramedullären Kavitationen beginnt für gewöhnlich caudal des
Epizentrum der Läsion und betrifft erst zu einem späteren Zeitpunkt kranielle Segmente, wenn es zur Ausdehnung der Läsion gekommen ist.


Zusammenfassend kann gesagt werden, dass der zeitliche Verlauf und die Charakterisierung von Rückenmarkstraumata erfolgreich durch DTI in einer homogenen Gruppe von Hunden mit akuten und chronischen Rückenmarksschädigungen bewertet werden konnten. Für Entwicklung und Durchführung von neuen Behandlungsstrategien bei Rückenmarksschädigungen stellt DTI eine praktische, nicht-invasive Methode zur
9. Chapter 9: References


Chapter 9: References

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Griffiths IR. 1972. Some aspects of the pathology and pathogenesis of the myelopathy caused by disc protrusions in the dog. J Neurol Neurosurg Psychiatry. 35(3):403-413.


10. Chapter 10: Annexes

10.1. Raw data: signalement, time from onset of non-ambulatory status to MRI and neurological status at admission.

Abbreviations:

DPP = Deep pain perception

+ = presence of DPP

- = absence of DPP

T2W-LER = T2-weighted - lesion extension ratio

N/A = not available

**Table 2.** Signalement, time from onset of non-ambulatory status to MRI and neurological status at admission of included dogs.

<table>
<thead>
<tr>
<th>Dog</th>
<th>State</th>
<th>Breed</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Body Weight (Kg)</th>
<th>Delay of clinical signs (days)</th>
<th>DPP</th>
<th>T2W-LER</th>
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<td>Weight (kg)</td>
<td>Sign</td>
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### 10.2. Raw data: diffusion tensor metrics obtained from the spinal cord of paraplegic dogs before undergoing surgical decompression.

**Abbreviations:**

- FA = fractional anisotropy
- ADC = apparent diffusion coefficient
- VB = vertebral body
- N/A = not available

#### Table 3. FA and ADC values from epicentres and one vertebral body cranially and caudally of included dogs before surgical decompression.

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10.3. Raw data: diffusion tensor metrics obtained from the spinal cord of dogs 3 months after functional motor recovery.

Abbreviations:

FA = fractional anisotropy

ADC = apparent diffusion coefficient

VB = vertebral body

N/A = not available

**Table 4.** FA and ADC values from epicentres and one vertebral body cranially and caudally of included dogs three months after showing motor function recovery.

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11. Chapter 11: Acknowledgements

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