Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado Integrado em Medicina Veterinária

Dissertação

Spinal Shock in Paraplegic Dogs and Neurorehabilitation

Natalina Isabel Cheang do Rosário Vong

Orientador(es) | Maria Teresa Oliveira
Maria Cristina Queiroga
Ângela Paula Neves Rocha Martins

Évora 2023

Esta dissertação não inclui as críticas e as sugestões feitas pelo júri.

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Abstract

The spinal shock (SS) phenomenon requires the need of implementing an early intensive neurorehabilitation protocol. This blinded cohort study, performed in Portugal, aims to observe the evolution of SS through the application of the Spinal Shock Scale (SSS) and its implications in the neurorehabilitation of dogs who suffered a spinal cord injury. 249 dogs were evaluated with the SSS, at the time of admission, and for every six hours for three days. All dogs underwent similar neurorehabilitation protocols including land and underwater treadmill training and functional electrical stimulation. Patients admitted under 48h post-injury (71.5%) had higher SSS scored due to early detection of SS signs. Estimated marginal means of SSS showed an exponential decrease in the first 6h, followed by a relative plateau, which was always faster in non-compressive myelopathy patients. The application of the SSS can be beneficial, mainly to adapt neurorehabilitation protocols to each patient's needs throughout treatment.

Keywords: Spinal Shock, SCI; Dogs; Neurorehabilitation; Spinal Shock Scale.

Resumo

Choque Espinhal em Cães Paraplégicos e Neuroreabilitação

O Choque Espinhal (CE) exige a implementação precoce de protocolos de neurorehabilitação intensiva. Este estudo coorte cego, realizado em Portugal, tem como objetivos observar a evolução do CE através da aplicação do *Spinal Shock Scale* (SSS) e as suas implicações na neurorehabilitação de cães que sofreram lesão medular. Foram avaliados 249 cães com o SSS, à admissão e a cada seis horas durante três dias. Foram todos submetidos a protocolos semelhantes, incluindo treino em passadeira terrestre, aquática e estimulação elétrica funcional. Pacientes admitidos antes das 48h pós-lesão (71.5%) tiveram pontuações mais elevadas na SSS devido à deteção precoce de sinais de CE. As médias marginais estimadas do SSS mostraram uma diminuição exponencial nas primeiras 6h, seguido de um plateau, sendo sempre mais rápido em pacientes com mielopatias não-compressivas. A aplicação do SSS pode ser benéfica para adequar os protocolos de neurorehabilitação às necessidades de cada paciente ao longo do tratamento.

Palavras-Chave: Choque Espinhal; Lesão medular; Cães; Neurorehabilitação; Spinal Shock Scale.

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Acronyms

3-NT	3-nitrotyrosines
ACVIM	American College of Veterinary Internal Medicine
AF	Annulus Fibrosus
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANNPE	Acute Non-Compressive Nucleus Pulposus Extrusion
BWSTT	Body weight supported treadmill training
Ca^{2+}	Calcium
CANSORT	Canine Spinal Cord Injury Consortium
CCRP	Certified Canine Rehabilitation Practitioner
CE	Choque Espinhal
CMAP	Compound Muscle Action Potential
CNS	Canadian Neurological Score
CPG	Central Pattern Generator
CR^2AL	Centro de Reabilitação e Regeneração Animal de Lisboa
CRAA	Centro de Reabilitação Animal da Arrábida
CSF	Cerebrospinal Fluid
CT	Computed Tomography
CVI	Content Validity Index
CVR	Content Validity Ratio
DPP	Deep Pain Perception
DPR	Delayed Plantar Reflex
ES	Electrical Stimulation
FCEM	Fibrocartilaginous Embolic Myelopathy
FES	Functional Electrical Stimulation
FGF4	Fibroblast Growth Factor 4
FNRS-DPN	Functional Neurorehabilitation Scale for Deep Pain Negative
GABA	γ-Aminobutyric acid
ICC	Intraclass Correlation Coefficient
IVD	Intervertebral Disc
IVDH	Intervertebral Disc Hernia
LLLT	Low Light Laser Therapy
LM	Lesão Medular
LMN	Lower Motor Neuron
LT	Locomotor training

MRI	Magnetic Resonance Imaging
Na	Sodium
NMDA	N-methyl-d-aspartate
NP	Nucleus Pulposus
NSAID	Non-Steroidal Anti-inflammatory Drugs
OFS	Open Field Score
PROM	Passive Range of Motion
RASS	Richmond Agitation-Sedation Scale
RNS	Reactive Nitrogen Species
ROM	Range of Motion
SCI	Spinal Cord Injury
SD	Standard Deviation
SEM	Standard Mean of Error
SPSS	Statistical Package for the Social Sciences
SS	Spinal Shock
SSS	Spinal Shock Scale
TENS	Transcutaneous Electrical Nerve Stimulation
TESCS	Transcutaneous Electrical Spinal Cord Stimulation
UMN	Upper Motor Neuron
UWTT	Underwater treadmill training
VAS	Visual Analog Scale

1. Introduction

Spinal shock (SS) is an under reported phenomenon in the veterinary field that, upon neurological examination, may lead to inaccurate lesion localization and, therefore, misdiagnosis due to its paradoxical nature. When one is not aware of SS, the differential diagnosis list may include and prioritize myelopathies with a much more reserved prognosis. For this reason, the author wanted to explore this phenomenon and, hopefully, bring some awareness so that others can recognize it as well.

1.1. Spinal shock

Spinal shock can occur after a spinal cord injury and it has been defined as a depression or absence of spinal reflexes as well as muscle tone caudal to the site of injury, despite the arc reflex remaining intact (Smith & Jeffery, 2005). The bladder can also be flaccid with sphincter hypotonia (Lorenz *et al*, 2011). It should be noted that spinal shock should not be mistaken by neurogenic shock although both terms have been used interchangeably in the past, the latter is a form of distributive shock being characterized by hemodynamic changes that occur subsequently to a spinal cord injury (Mack, 2013).

During the neurologic exam, it is required to assess postural reactions as well as segmental spinal reflexes in order to neuroanatomically localize the lesion site. When reflexes are depressed or absent, it implies that the lesion is localized within the arc reflex, which is known as a lower motor neuron (LMN) lesion, whereas intact spinal reflexes but depressed postural reactions indicate an upper motor neuron (UMN) lesion. When the exam does not reveal a solitary lesion site, a multifocal process is assumed (Smith & Jeffery, 2005).

Often times, diminished pelvic limb reflexes is evocative of a L4-S3 segments lesion, however these reflexes can also be diminished in T3-L3 myelopathies associated with SS, this is why the presence of this phenomenon can lead to an inaccurate neuroanatomic localization of the lesion site since it is an UMN lesion but shows signs of a LMN lesion. The clinical presentation of a T3-L3 myelopathy associated with SS can be confused with LMN lesions, such as progressive myelomalacia or a multifocal inflammatory myelopathy, which have a more reserved prognosis. In these cases, one should always request a magnetic resonance imaging (MRI) or a computed tomography (CT) imaging of the T3-L3 and L4-S3 segments, in order to avoid misdiagnosis (McBride, 2021).

SS is usually temporary and particularly short-lived in domestic animals, lasting a few minutes to hours. It starts with a period of areflexia, where there is an absence of spinal reflexes, followed by a period of normal muscle tone and reflex response and finally hypertonia (Lahunta, 2009). In an experimental environment, Blauch (1977) observed that after a spinal cord transection in dogs, the anal sphincter reflex comes back in 15 minutes, followed by the patellar reflex after 30 minutes to two hours, and the flexor withdrawal reflexes need up to 12 hours (Blauch 1977 cit. by Smith & Jeffery, 2005). However, Hodshon and Thomas' (2018) study revealed that this last reflex can take between 1.5 to 6.5

days to recover (Hodshon & Thomas, 2018), while other authors argue that it may take from two days to six weeks (Full *et al*, 2016). In domestic animals, spasticity starts developing in 24 to 48 hours (Smith & Jeffery, 2005), whereas in primates the depression of reflexes will last for two to three weeks (Lahunta, 2009).

1.1.1. Pathophysiology of Spinal Shock

The underlying mechanism of SS still remains unknown, some hypothesize that it occurs with neurochemical alterations, mainly the increase of glycine, a major inhibitory neurotransmitter, fusimotor depression or by hyperpolarization of spinal motor neurons which is backed by Sherrington's research (Smith & Jeffery, 2005; Ko, 2018; McBride, 2021).

Sherrington (1947) suggests that SS develops below the spinal cord transection and is caused by the halt of descending supraspinal input (Sherrington, 1947). After a spinal cord transection, spinal reflexes become absent due to the lack of synaptic transmission and interneuron conduction. This causes a hyperpolarization of the spinal motor neurons, thus a reduction of their excitability (Smith & Jeffery, 2005). This theory was later explored by McCouch's (1966) experiments in cats and monkeys in which a unilateral lesion was made in only one descending tract, followed by a period of reflex recovery, and a subsequent full transection of the spinal cord. McCouch (1966) then followed the reflex recovery from both sides and found that the reflexes from the initial paretic limb recovered faster than when full transection was made. This not only supports Sherrington's theory that SS is caused by the interruption of descending input (Nacimiento & Noth, 1999), but could also mean that likely SS is as severe as the number of descending tracts that are interrupted (Bach-y-Rita & Ilis, 1993). It was also observed that reflex recovery occurred much faster in cats than monkeys (Chambers *et al.*, 1966), which is explained by the anatomical differences explored later on.

In the mammalian central nervous system, some amino acids behave as neurotransmitters. Glycine, the most abundant neurotransmitter in the spinal cord, as well as taurine and γ-aminobutyric acid (GABA), are considered inhibitory neurotransmitters, whereas glutamate and aspartate function as excitatory neurotransmitters on the segmental motor neurons. Simpson has observed that in rabbits, during an experimentally induced SS period, levels of glycine were about two to three times higher than its baseline, which did not happen with the other neurotransmitters, aspartate, glutamate, or taurine. The increase of glycinergic activity has shown to decrease muscle tone, more so, cases of congenital and iatrogenic hyperglycinemia are expressed by severe hypotonia and flaccidity. On the other hand, the use of a glycine receptor specific antagonist, strychnine, has shown an increment of muscle tone. With Simpson's (1966) investigation, it could be suggested that glycine may, possibly, have a role in the areflexia phase of spinal shock (Simpson *et al*, 1996).

Another contributing hypothesis for the absence of reflexes, includes the depression of the fusimotor system's activity (γ motor neurons). This system regulates the sensitivity of muscle tone and reflexes. As the γ motor neurons regulate muscle spindle tension, when there is loss of the descending input there's also reduced muscle spindle excitability, therefore, there will be decreased segmental input to the afferent motor neurons (Hodshon & Thomas, 2018; Ko, 2018).

Neuroplasticity, that is, the capacity to regenerate or reorganize of the mature central nervous system as well as its capacity to relearn, are what allows the recovery of the reflexes after a spinal cord injury. Denervated motor neurons become hypersensitive allowing an increased response to a certain stimulus. This is hypothesized to be caused by the rise of availability of excitatory neurotransmitters as a result of spent neurotransmitter not being taken back to nerve terminals as in a normal physiologic process, and the possible modification of the receptors so that production is increased, degradation decreased, and subunits modified to have a greater receptivity of remaining input (Smith & Jeffery, 2005).

The development of hyperexcitability or spasticity is characterized by not only an increased reflex response and muscle tone, but also involuntary muscle contractions (Hiersemenzel *et al*, 2000). This is speculated to happen through the growth of new excitatory synapses on motor neurons caudal to the lesion. These new synapses are possibly derived from both spinal interneurons and primary segmental afferent neurons. These new afferent fibers could adhere to previously inhibitory synapses, turning them into excitatory ones (Sheean, 2002). Physiologically, motor neurons receive input from the supraspinal tract, interneurons, and primary segmental afferent fibers, thus when the supraspinal information is interrupted, local neurons can form new synapses. Nevertheless, the time span during which these synapses are formed depends on the length of the axons. This could explain why there is a normal reflex response phase and then a hyperreflexia phase – firstly, there are new synapses coming from the interneurons, since their body cells are closer to the terminal axons, and later, others from the primary sensory afferent axons, which causes spasticity (Ditunno *et al.*, 2004; Smith & Jeffery, 2005).

1.1.2. Spinal shock differences in human and domestic animals

Spinal shock has been described in humans since the 1800. Initially, the term was used in an attempt to differentiate arterial hypotension secondary to hemorrhage from arterial hypotension due to loss of sympathetic feedback caused by a spinal cord injury (SCI). Later on, this term was used to define a permanent loss of tendon reflexes, muscle tone, and sensory functions below a spinal cord lesion. Throughout the years, with patient care and research, SS's concept developed into what is defined today, changing the term "permanent" into "temporary" loss of reflexes and muscle tone below the site of injury (Atkinson & Atkinson, 1996).

SS affects each species differently. It is reported to be more profound and longer lasting in higher primates, especially humans, ranging from days to weeks, and averaging to around four to 12 weeks. Since the recovery is so drawn out, Ditunno *et al* (2004) were able to define and separate it into four different stages:

1. Phase One (first day post-injury) – areflexia or hyporeflexia

Clinically, this initial phase is characterized by muscle hypotonia and the absence of reflexes, such as the ankle and the patellar reflexes, caudal to the site of injury (Ditunno *et al.*, 2004). Usually, the first reflex to appear is the delayed plantar reflex (DPR), which is a pathological reflex and can be elicited hours after the injury (Ko *et al.*, 1999). Cutaneous reflexes, such as the bulbocavernosus, the cremasteric reflexes, and the anal wink, start restoring at this phase (Ko, 2018).

It is hypothesized that, after the SCI, spinal neurons become less excitable, leading to the reflex depression, and the γ motor neurons, which are responsible for maintaining muscle spindle tension, loses the descending tonic facilitation, causing muscle hypotonia (Ditunno *et al.*, 2004).

2. Phase Two (first to third day post-injury) – reflex return

In this phase, the cutaneous reflexes discussed above become stronger. Normally, deep tendon reflexes are not present yet, however, in older patients, these reflexes and the Babinski reflex can be present (Ko *et al.*, 1999).

This initial reflex recovery is thought to be due to denervation supersensitivity and receptor upregulation (Ko, 2018). This is a compensatory neuroplasticity phenomenon that occurs when there is no signal passing through a damaged neuron (O'Reilly *et al.*, 2019). It is thought to occur with four different mechanisms: the post-synaptic membrane produces more receptors for neurotransmitters and decreases its removal/degradation, there is reduced reuptake of excitatory neurotransmitters, and the receptor subunits is altered. This increase in excitatory neurotransmitters and receptor plasticity may explain the resurgence of the cutaneous reflexes (Ditunno *et al.*, 2004).

3. Phase Three (first to fourth week post-injury) – initial hyperreflexia

During this period, most deep tendon reflexes recover. The ankle jerk is the first to recover, followed closely by the Babinski reflex and, later on, the patellar reflex (Ko *et al.*, 1999). All cutaneous reflexes are usually present by the end of this period. The DPR will persist beyond a month in only 10% of the cases (Ditunno *et al.*, 2004).

During this phase, new synapse growths occur. They can be originated from spinal interneurons and primary segmental afferents. This growth is thought to be dependent on the axon length, which

can explain why the H-reflex excitability and hyperreflexia occurs in a biphasic manner. Spinal interneurons have shorter axon lengths while the segmental afferent have longer axons lengths. It is thought that the initial synapse growth occurs in the shorter interneurons, which will provide the cutaneous reflexes, followed by growth from the longer primary afferents, which will relay the H-reflex (Ditunno *et al.*, 2004; Ko, 2018).

4. Phase Four (first to 12th month post injury) – final hyperreflexia

During this fourth stage of SS, DPR is absent in most patients. Cutaneous, deep tendon, and the Babinski reflexes have all become hyperresponsive to a minimal stimulus (Ditunno *et al.*, 2004).

The mechanisms for this phase are identical to the ones discussed on phase three. If there is insignificant sparing of the descending motor input, the new synapses from segmental reflex inputs can cause undesirable results such as spasticity and limited voluntary movement, moreover, patients with an incomplete SCI and preserved descending motor input can have a functional recovery (Ditunno *et al.*, 2004).

In humans, the Babinski reflex is a noxious superficial reflex which is elicited by running a long, blunt object from the lateral aspect of the foot's surface through the transverse arch and stopping at the middle metatarsophalangeal joint (Ambesh *et al.*, 2016). A positive Babinski reflex, this is, the dorsiflexion of the foot, extension of the big toe with fanning of the toes, is associated with a pyramidal tract lesion (Jaramillo *et al.*, 2014). If the corticospinal (pyramidal) tract is intact, the normal response to the noxious stimulus of the foot's sole should be flexion of the toes. It should be noted that this reflex occurs physiologically in newborns and disappears as their corticospinal tract develops (Ambesh *et al.*, 2016).

The recovery in non-primates is much quicker, ranging from a few minutes to days. According to Blauch (1977), the anal sphincter reflex in dogs is recovered after 15 minutes, followed by the patellar reflex between 30 minutes to two hours post-injury (Blauch 1977 cit. by Smith & Jeffery, 2005). Hyperreflexia starts developing within 24-48 hours. Due to the fast progression of SS in domestic animals, it is rare to be able to detect phases one and two of SS. Most animals will be presented in late phase three or beginning of phase four. This aspect could be due to the anatomic differences that exist in the descending motor control. In humans, the corticospinal tract is well developed and gives the motor cortex a direct influence on the motor neuron activity. Its axons originate from the motor cortex of the frontal lobe, run ipsilateral to the midbrain and medulla and 85-90% of the fibers decussate in the pyramid (Fig. 1). These crossing axons are known as the "lateral corticospinal tract", and they are responsible for the refined conscious control of the limbs and digits. The other smaller percentage of axons that do not decussate form the "ventral corticospinal tract" and it controls the proximal postural muscles (Cunningham & Klein, 2007).

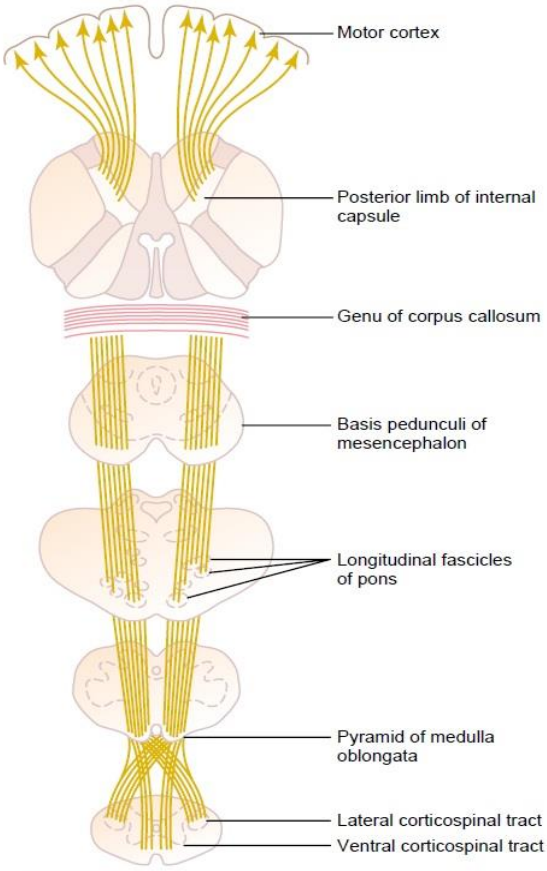

Fig. 1: The Corticospinal tract (Modified from Ranson & Clark, 1959) Source: https://www.brainkart.com/article/Transmission-of-Signals-from-the-Motor-Cortex-to-the-Muscles_19731/

On the other hand, in non-primates, the underdeveloped corticospinal tract has an insignificant role to locomotion. The little input it does provide is indirect, through synapses in the brainstem nuclei or through interneurons in the spinal cord (McBride, 2021) thus, the role for non-primates' mobility is taken up by the extrapyramidal tracts (rubrospinal, reticulospinal and vestibulospinal). The extrapyramidal tracts originate from the brainstem and travel within the ventral and lateral funiculi of the spinal cord. They are also thought to communicate with pelvic limbs' LMN, therefore, the lack of their input likely results in the development of SS with paraplegia or paraparesis when an injury occurs (McBride *et al.*, 2022), but the lack of corticospinal input does not seem to contribute to SS (McBride, 2021). As discussed above, motor neurons can recover their function through denervation supersensitivity of the other pre-existing spinal reflex circuits, as well as, by the growth of new synapses. In humans, if the SCI destroys the whole corticospinal tract, the interneuron plasticity in this specific tract will not be possible, thus the recovery will be longer (Smith & Jeffery, 2005).

1.2. Diseases commonly associated with spinal shock

According to the American College of Veterinary Internal Medicine (ACVIM) and the Canine Spinal Cord Injury Consortium (CANSORT-SCI), as well as three different studies, SS is associated with SCI, mainly acute intervertebral disc herniation (IVDH), acute non-compressive nucleus pulposus extrusion (ANNPE) and fibrocartilaginous embolic myelopathy (FCEM) (Full *et al*, 2016; Hodshon & Thomas, 2018; McBride, 2021). In humans, it has been reported that more than 90% of all SCI are due to trauma, including traffic and sport accidents and falls (Alizadeh *et al*, 2019).

1.2.1. Trauma

In human medicine, patients presenting with history of a traumatic SCI should be stabilized and any life-threatening issues will need to be addressed (Jia *et al.*, 2013). As these patients can be in neurogenic shock, close monitoring should be kept on their systemic blood pressure and oxygen saturation, in order to avoid ischemia and further aggravation of secondary injury (Eckert & Martin, 2017), as discussed later on. For domestic animals, spine immobilization should be assured with the patient in lateral recumbency anytime the vertebral column's integrity is in question or there are suspicions of vertebral fractures (Park *et al*, 2012).

Once the patient is stable, imaging can be performed to be able to rule out vertebral fractures or luxations (Wang *et al.*, 2021). While radiographs are useful for detecting gross fractures, CT is proved to be the best method for bone imaging thus, evaluating the severity of vertebral fragments and the presence or not of bone fragments in the vertebral canal. On the other hand, MRI is the best suited technique to evaluate the surrounding soft tissues, such as intervertebral discs, ligaments, nerve roots and the spinal cord itself (Park *et al*, 2012; Ahuja *et al.*, 2017).

1.2.1.1. Primary injury

Traumatic spinal cord injury occurs in a biphasic manner. The first phase, known as the primary injury, takes place when there is an external acute physical force on the spinal cord, which disrupts the vertebral column causing compression or, more rarely, transection of the spinal cord (Ahuja *et al.*, 2017). There are four main mechanisms for primary injury: the most common being impact with persistent compression, which is frequently associated with vertebra fractures or subluxations that will compress the spinal cord. Less commonly reported are impact with transient compression, which are normally associated with hyperextension injuries; distraction injuries that refer to when the spinal cord is stretched in the axial plane; and laceration injuries that arises when sharp bone fragments tear through the tissue within the spinal cord. Lacerations can vary in the degree of injury, ranging from minor injury to complete transection (Park *et al*, 2012; Alizadeh *et al*, 2019).

The initial primary injury, regardless of its form, will compromise spinal cord's microvasculature, damage cell membranes and the ascending and descending tracts in the spinal cord (Ahuja *et al.*, 2017; Alizadeh *et al*, 2019), which in turn will trigger the onset of a complex cascade of molecular and biochemical events known as the secondary injury, which will further damage the spinal cord and its neurological functions (Rowland *et al.*, 2008).

At this point, treatment for the primary injury relies on preventing the aggravation of the spinal cord's compression by realigning the vertebral column and reducing instability (Shores, 1992; Bagley, 2000). Depending on its severity, this can be done by immobilization alone with external coaptation or pursuing decompressive surgery (Park *et al*, 2012). Conservative management of a vertebral fracture or luxation is indicated in animals that present with minimal pain, little to no neurological deficits, and/or imaging shows next to no instability (Jeffery, 2010).

In order to classify spinal fractures and determine the degree of the vertebral column's stability, the "three-compartment system" was adapted from human medicine to be used in small animals (Shores, 1992). This system divides the vertebra into three compartments, the dorsal compartment that comprises of the spinous process, articular facets, *pediculi*, laminae and the *ligamentum flavum*; the middle compartment consisting of the dorsal vertebral body, dorsal *annulus*, and the dorsal longitudinal ligament; finally, the ventral compartment which contains the rest of the vertebral body, the *nucleus pulposus* (NP), the lateral and ventral aspects of the *annulus* and the ventral longitudinal ligament (Fig. 2) (Shores, 1992). This concept states that if two or more of these compartments appear damaged or luxated in the imaging, the fracture is regarded as unstable and surgical intervention should be indicated (Bagley, 2000; Jeffery, 2010).

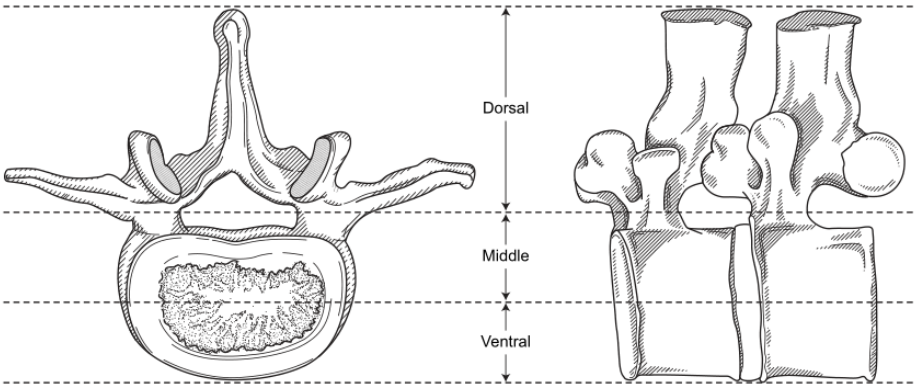

Fig. 2: "Three-compartment system" developed for assessment of the vertebral column's stability. (Source: Jeffery, 2010)

1.2.1.2. Secondary injury

A. Immediate or acute phase

The secondary injury begins after the primary injury and can last from days to weeks. Its phases can be divided temporally in acute or immediate (first 48 hours), sub-acute (48 hours to 14 days), intermediate (14 days to six months) and chronic phases (after six months) (Ahuja *et al.*, 2017). The acute phase directly reflects the results of the primary injury, mainly the neurogenic shock and disruption of the vasculature, leading to hemorrhage and hypovolemia, consequently causing ischemia due to the compromised spinal cord perfusion and cellular death, which will offset the inflammatory cascade, resulting in edema that will, ultimately, worsen the injury. Following the ischemic period, comes the reperfusion stage that, paradoxically, will aggravate injury, as it causes more cellular death due to the release of oxygen and nitrogen free radicals. In parallel, cellular death and ischemia will lead to an increase of extracellular glutamate, that will offset excitotoxicity (Dumont *et al.*, 2001; Ahuja *et al.*, 2017; Alizadeh *et al*, 2019).

B. Sub-acute phase

In the sub-acute phase, as a result of cellular damage as well as ischemia, there is an increase in glutamate, a central excitatory neurotransmitter. This neurotransmitter binds to inotropic receptors, mainly N-methyl-d-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors, as well as metabotropic receptors, excessively activating them, resulting in an influx of calcium (Ca^{2+}) and sodium (Na^{+}) into the cells. On one hand, sodium uptake will cause cytotoxic edema, on the other, the high intracellular Ca^{2+} will, not only cause cell death by excitotoxicity, but also, by a complex cascade of reactions, generate oxygen and nitrogen free radicals that will provoke oxidative stress (Dumont *et al.*, 2001; Ahuja *et al.*, 2017; Couillard-Despres *et al*, 2017).

As mentioned before, oxygen and nitrogen free radicals have been released, not only due to ischemia-reperfusion lesion, but also as a result of intracellular Ca^{2+} influx, leading to oxidative stress, mainly lipid peroxidation and protein oxidation. Lipid peroxidation is a free radical reaction that causes oxidation of the cell membrane lipids, resulting in cell lysis (Rowland *et al.*, 2008), while protein oxidation occurs when reactive nitrogen species (RNS) nitrate tyrosine, present in amino acids, resulting in 3-nitrotyrosines (3-NT), an important biomarker for protein damage (Couillard-Despres *et al*, 2017). Overall, lipid and protein oxidations cause severe damages at a cellular level that ultimately will lead to cellular death (Alizadeh *et al*, 2019).

C. Intermediate phase

As the initial damage subsides, the lesion will start to stabilize and evolve, on one hand, with the formation of glial scar and cystic cavitations, and on the other, with attempts on remyelination and axonal regeneration (Ahuja *et al.*, 2017). Glial scar is the scar tissue that forms around the lesion site after the SCI, consisting of predominantly activated astrocytes that have hypertrophied. Although the glial scar aids in the reestablishment of both the physical and chemical integrity of the spinal cord, closes the blood-brain barrier, and serves as a protective layer preventing immune cells to spread to adjacent segments, it showed that it also limits the ability of the spinal cord to repair and regenerate itself (Alizadeh *et al*, 2019). Similarly, as the macrophages removes all the dead cells, it leaves behind cystic cavities that limits axonal regeneration (Ahuja *et al.*, 2017). In addition, in cases where the meninges are also damaged, fibroblasts will infiltrate the lesion, further contributing to this restrictive setting (Couillard-Despres *et al*, 2017).

D. Chronic phase

Chronic phase starts around six months post injury and according to Rowland *et al* (2008), it is only after one to two years post-injury that the lesion and subsequent functional deficits are considered stabilized. During this phase, the glial scar continues to mature, and axon regeneration continues to take place. The Wallerian degeneration, this is, the progressive degeneration of the distal portion of the injured axon, persists and could take multiple years for the damaged axons and its cell bodies to be completely eliminated (Rowland *et al.*, 2008).

The cystic cavitations that started to arise during the previous phase will potentially cause disturbances of the cerebrospinal fluid (CSF) flow. In one third of human patients that have suffered a SCI, this phenomenon will lead to the development of syringomyelia, which is a source of neuropathic pain and further contributes to neurologic deficits (Couillard-Despres *et al*, 2017). According to Falci *et al* (2009), surgically duroplasty expansion and cyst shunting can be effective in reducing this neuropathic pain (Falci *et al*, 2009).

1.2.1.3. Post-injury management

Post-injury supportive care includes pain management, commonly using opioids, management of bed sores, by frequent switching of recumbencies, and bladder management, as many of these patients might have bladder dysfunction (Park *et al*, 2012). Physical rehabilitation is also crucial for maximizing the neurological functional recovery as it aids in the neuroregeneration process. In human medicine, patient improvement is correlated with the degree of activity that is tolerated by them, therefore physical therapy is recommended as early as day one post-injury, aiming for at least 20 minutes of tolerated activity per

day (Wang *et al.*, 2021). Additionally, starting locomotor training (LT) in the early post-injury stages has shown to accelerate functional recovery in those suffering from an incomplete SCI (Piira *et al.*, 2019), as it prevents muscle atrophy, enhances sprouting of spared descending circuits, proprioceptive formation, and plasticity mechanisms below the injury site, all of which are essential for functional recovery (Yu *et al.*, 2019).

Electrical stimulation (ES) is a neuromodulation method that improves neurologic function by stimulating neuronal networks below the injury site, increase these networks' excitability, and is thought to promote axonal growth (Jack *et al.*, 2020). ES can be used in a multimodal approach, that is, combining it with LT, such as body weight supported overground walking or treadmill training, and kinesiotherapy exercises. The use of this approach showed greater capacity to promote reorganization and strengthening of the neural networks for locomotion, thus improving functional recovery (Ragnarsson, 2008; Rowland *et al.*, 2008; Ahuja *et al.*, 2017; Yu *et al.*, 2019).

1.2.2. Intervertebral disc herniation

1.2.2.1. Hansen type I

Intervertebral disc herniation consists of a localized displacement of part of the intervertebral disc (IVD), generally, into the vertebral canal. Hansen type I or acute intervertebral disc extrusion is usually associated with IVD degeneration known as chondroid metaplasia, a progressive dehydration, loss of glycosaminoglycans and increase of collagen content of the NP, which is normally gelatinous, hydrated, and rich in notochordal cells, resulting in the loss of hydroelasticity of the disc and its ability to sustain pressure, ultimately leading to the extrusion of the degenerated NP through the ruptured *annulus fibrosus* (AF) into the vertebral canal (Brisson, 2010; Fenn *et al*, 2020).

This affects breeds that have an altered endochondral ossification with shortened long bones, known as chondrodystrophic breeds, particularly Dachshunds, French Bulldogs and Pekingese, due to an expressed retrogene, known as fibroblast growth factor 4 *(FGF4),* in a locus on chromosome 12, which is associated with disc calcification and IVDH (Brown *et al.*, 2017; Murphy *et al.*, 2019). According to Brisson (2010), chondroid metaplasia can begin as early as two months in Dachshunds and by the age of one, chondrodystrophic dogs have 75-90% of their gelatinous NP replaced into a more cartilaginous tissue. Around 24-90% of Dachshunds have one or more mineralized IVD (Brisson, 2010).

Hansen type I most commonly affects young to middle-aged chondrodystrophic dog breeds, but it can also affect non-chondrodystrophic breeds, as well as cats. It can happen anywhere along the vertebral column, although 75% are found between T12-L2. Cervical level herniations are less common, making up approximately 14-35% of all IVDH (Spitzbarth *et al.*, 2020). The clinical presentation is generally an acute, painful, progressive myelopathy. Depending on the location of the extrusion, clinical signs can range from

discomfort, with no neurological deficits, to paralysis of the affected limbs, with no deep pain perception (DPP) (Fenn *et al*, 2020).

Diagnosis can be obtained by MRI or CT. Although plain radiographs or myelography (Fig. 3) can be done, their findings can only be indicative of IVD extrusion, but never a diagnostic method, as it only has a reported sensitivity of 51-61% in the thoracolumbar region and even lower in the cervical region (Costa *et al.*, 2020). Radiographic findings include reduction of the affected disc's space and mineralized disc material present in the vertebral canal. CT (Fig. 4) and MRI (Fig. 5) are considered far better diagnosis methods. The latter is the gold standard as it can be used to evaluate soft tissues surrounding the herniation, namely the spinal cord and intervertebral discs, and it is the only tool capable of diagnosing non-mineralized foraminal or lateral extrusions (Fenn *et al*, 2020).

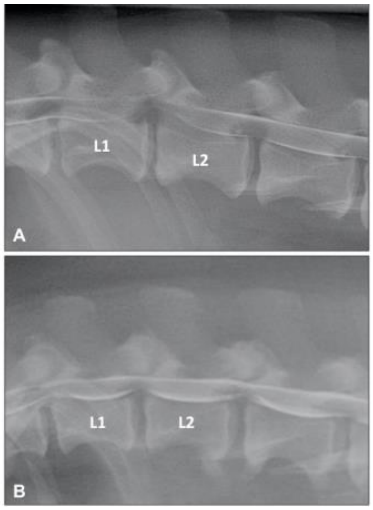

Fig. 3: Lateral radiograph with myelography of a IVD extrusion in L1-L2. A: Severe thinning of the contrast columns which appears to be nearly discontinued at L1-L2. B: Lateral radiograph with myelography with IVD protrusion between L1-L2 and L2-L3. (Source: Costa et al, 2020)

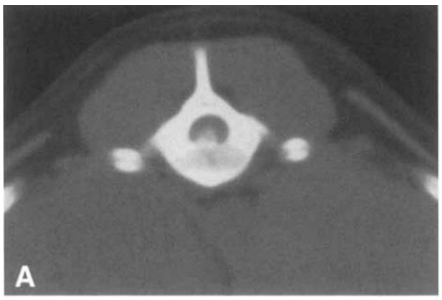

Fig. 4: Computed tomography image of a mineralized IVD extrusion in a caudal thoracic vertebra. A: Hyperattenuating mass present in the ventral aspect of the vertebral canal causing compression of the spinal cord. (Source: Olby et al, 2000).

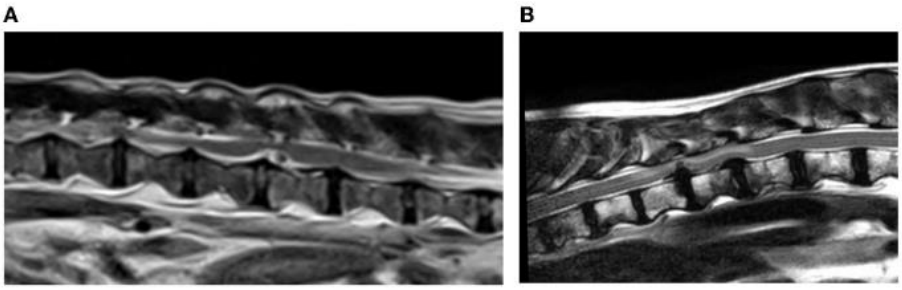

Fig. 5: Sagittal MRI of intradural/extramedullary IVD extrusions. A: Sagittal imaging showing intradural/extramedullary IVH extrusion in L3-L4; B: Sagittal imaging showing intradural/extramedullary IVH extrusion in T11-T12. (Source: Casado et al, 2022)

1.2.3. Hansen type II

IVD protrusion or Hansen type II, is normally associated with fibroid metaplasia, where the notochordal cells of the NP slowly become more like fibrocytes and there is an increment of collagen material. It is mainly observed in non-chondrodystrophic breeds over the age of seven and consists of a shift of the NP as a result of a partial breach and weakening of the AF, possibly due to repeated minor trauma, leading to a focal protrusion of the NP on the surface of the AF dorsally, into the vertebral canal, slowly compressing the spinal cord. Unlike chondroid metaplasia, fibroid metaplasia affects only a small number of discs and mineralization is uncommon (Brisson, 2010; Fenn *et al*, 2020).

Clinical presentation will depend on the location of the affected IVD as well as the level of compression of relevant structures, such as the spinal cord and nerve roots. As it is a chronic, progressive disease, its clinical signs will also be of a slow progressive, non-painful myelopathy, in an older, generally non-chondrodystrophic dog, typically with mild neurological deficits. Although pain is less common than in Hansen type I, it can also be present if there's nerve root compression. As plain radiographs cannot be used as a diagnosis method for IVD protrusion, seeing that it only shows non-specific signs of IVD degeneration such as vertebral endplate sclerosis, spondylosis deformans and IVD narrowing, the diagnosis is made with MRI (Costa *et al.*, 2020). Myelography has been a useful tool for differentiating IVD extrusion from IVD protrusion through the criteria present in Table 1:

Table 1: Criteria for myelography IVD extrusion and IVD protrusion differentiation (adapted from Costa *et al*, 2020)

IVD extrusion	**IVD protrusion**
Thinning and deviation of the contrast columns	Thinning and deviation dorsal or dorsolateral of the contrast columns
Thinning of the contrast columns is mild to severe or discontinuous	Thinning of the contrast columns is mild
Diffuse thinning of the contrast columns and beyond the boundaries of the affected disc	Focal and centered thinning of the contrast columns cranial or caudally to the affected disc
Asymmetrical distribution of the contrast column thinning cranial or caudally to the affected disc	Symmetrical distribution of the contrast of the column thinning cranial or caudally to the affected disc

3.2.3. Treatment of intervertebral disc herniations

Depending on the patient's clinical signs and presentation, treatment can be conservative or surgical (Gouveia *et al.*, 2022b). Conservative treatment consists of cage rest for two to six weeks to allow the AF to heal and to avoid worsening of the extrusion. The administration of anti-inflammatory drugs (NSAIDs), analgesics, and muscle relaxants have been used, but it is still unclear whether it provides benefits or not. This type of treatment is more adequate to patients with an acute onset and mild clinical signs. In cases where conservative treatment does not work, chronic patients or patients that show severe pain and neurologic deficits, surgical decompression treatment should be pursued. Techniques such as ventral slot, dorsal laminectomy, and hemilaminectomy have been described for the resolution of cervical herniations. It should also be noted that conservative treatment is contraindicated in patients with no deep pain perception (DPP) (Brisson, 2010).

Similar to cervical herniations, the aim for surgical treatment of thoracolumbar herniations is decompression. According to Moore *et al* (2020), the surgical technique of choice for neurologists and surgeons is hemilaminectomy with or without removal of the articular process, as it provides adequate spinal cord decompression (Moore *et al.*, 2020). Less commonly, minimal invasive techniques such as mini-hemilaminectomy, pediculectomy, dorsal laminectomy with preservation of the articular process were also reported (Moore *et al*, 2016). Since these techniques are less invasive and remove less vertebral bone, they cause less vertebral instability, less tissue trauma, and consequently a faster recovery. The removal of extruded disc material is recommended both in patients with severe or declining neurologic deficits and patients with minimal neurologic deficits or back pain (Brisson, 2010).

Following surgical treatment, a physical rehabilitation protocol should also be implemented to maximize functional neurological recovery. These protocols may include locomotor training such as body

weight supported treadmill training (BWSTT), underwater treadmill, electrical therapy, and kinesiotherapy exercises (Gouveia *et al.*, 2022b). The repetitive stepping movements of locomotor training has shown that it improves coordinated rhythmic activity by activating central pattern generators (CPG) present in the spinal cord, in addition to promoting muscle strength, which is important for posture and gait, and stimulating spared ascending and descending tracts, therefore showing positive effects in regaining the ambulatory status (Martinez *et al.*, 2012; Martins *et al.*, 2021a). Kinesiotherapy exercises, such as bicycle movements, postural standing, balance board and walking in different floor surfaces, when used alongside this modality, help to correct imbalances and enhance synaptic neuroplasticity (Martins *et al.*, 2021c).

The aim of electrical therapy, such as Functional Electric Stimulation (FES), is to produce muscle contraction, therefore strengthening pelvic limb muscles, and stimulating the regeneration and activation of new neural circuits. On the other hand, Transcutaneous Electrical Spinal Cord Stimulation (TESCS) is thought to activate spinal networks, by increasing depolarization of descending motor tracts, while converging with ascending sensorial tracts. Moreover, TESCS is also thought to stimulate spared spinal tracts, as well as, aid in the generation of new ones (Martins *et al.*, 2021d).

1.2.3. Acute non-compressive nucleus pulposus extrusion

Acute non-compressive nucleus pulposus extrusion is described as a spinal cord contusion, with an acute extrusion of non-degenerated NP resulting in little to no compression. It occurs when the IVD is exposed to higher biomechanical forces such as blunt trauma or a brief period of intense exercise, causing a tear in the AF, and consequent extrusion of the nuclear material. As the hydrated NP impacts the spinal cord before being reabsorbed, it leads to a contusive spinal cord injury, but does not show signs of compression. It can be described along with history of trauma, but most commonly it has been associated with intense exercise (Gouveia *et al.*, 2022c). Clinical signs are usually of a non-progressive, non-painful, acute myelopathy, even though vocalization is often reported by the owners. Around 90% of dogs come with lateralized paresis or paralysis signs, whereas most cats are presented with symmetrical signs, albeit there are limited reports of feline ANNPE. On palpation, it reveals mild to no spinal hyperesthesia (Fenn *et al*, 2020).

ANNPE is usually diagnosed presumptively through MRI findings and characteristic medical history, since the definitive diagnosis can only be made post-mortem, through histological observation of the affected spinal cord segment, which reveals tears in the AF, non-degenerated NP material in the vertebral canal, as well as, a contused spinal cord (Risio *et al.*, 2009). The most affected segments are recorded to be the T3-L3 spinal cord segment, especially T12-L2 in dogs. This junction consists of a mobile lumbar segment and a relatively immobile thoracic segment, which causes strong biomechanical forces to act

upon it during exercise, being the reason why there is a higher frequency of ANNPE in this region (Risio, 2015).

ANNPE does not recall for surgery (Wessmann & Posporis, 2017) and is treated conservatively through anti-inflammatory and analgesic medications, if patients have spinal hyperalgesia.

Prognosis becomes poor when patients lose deep pain perception (DPP), develop symmetrical clinical signs, the lesion affects the intumescence, or if it affects a large area of the spinal cord (Fenn *et al.*, 2016; Gouveia *et al.*, 2022c).

1.2.4. Fibrocartilaginous embolic myelopathy

Fibrocartilaginous embolic myelopathy (FCEM) is a vascular condition in which fibrocartilaginous material, which is identical to the NP material of the IVD, gets embolized into the vasculature from the spinal cord and the leptomeninges. This can affect both the arterial and the venous side of circulation leading to ischemic necrosis of the affected segment of the spinal cord, resulting in an acute lateralized paresis or paralysis (Risio, 2015; Fenn *et al.*, 2016; Fenn *et al*, 2020). The main differential diagnosis of FCEM is ANNPE discussed above.

Large and giant non-chondrodystrophic breeds, frequently Labrador retrievers and Staffordshire bull terrier, have been the most reported breeds to be diagnosed with FCEM (usually body weight is >20kg), even though it has likewise been described in small breed dogs, especially in Miniature schnauzers. Mean age of diagnosis is approximately five to six years, and male:female ratio is roughly 2.5:1 in two different studies (Hawthorne *et al.*, 2001; Risio, 2015). The most reported breed in cats has been the domestic short hair, male:female ratio is 1.1:3 and median age is recorded to be 10 years (Theobald *et al.*, 2012).

FCEM is often diagnosed presumptively based on the characteristic clinical signs, medical history, and through MRI, which is the imaging method of choice. Definite diagnosis of FCEM can only be done post-mortem through the histological confirmation of the presence of fibrocartilage in the vasculature of the affected spinal cord segments (Theobald *et al.*, 2012).

Most patients present a non-painful and non-progressive acute onset, usually with asymmetrical neurological deficits. Owners often describe the onset of the neurological deficits during some type of physical exercise, and unlike ANNPE, spinal hyperalgesia is uncommon in FCEM. When diagnosis is done ante-mortem, the majority of the affected segments in dogs included L4-S3 and T3-L3 segments (Risio & Platt, 2010), while in cats the most affected segment is C6-T2 (Theobald *et al.*, 2012).

Like ANNPE, FCEM is treated conservatively through supportive care, physical rehabilitation and by maintaining adequate spinal cord perfusion. Physical rehabilitation is imperative for the recovery of

patients, especially those with severe neurological deficits. The prognosis will depend on the extent of the ischemic injury but is overall favorable. According to Risio & Platt (2010), negative prognostic elements include loss of DPP, LMN signs, symmetric neurologic deficits, severe neurological signs at the initial exam, owner's reluctance to provide nursing care and physical rehabilitation, as well as, if there is no improvement in the first 14 days (Risio & Platt, 2010).

Spinal shock usually doesn't happen in slow developing SCIs. Therefore, Hansen type I IVD herniation, ANNPE and FCEM, are more commonly associated with SS, due to their acute onset of clinical signs (Olby *et al.*, 2020).

1.3. Spinal shock as a prognostic factor

According to Mari (2019), dogs presented with ANNPE and spinal shock were two times more likely to develop faecal incontinence than those presented with ANNPE without SS, however it does not seem to affect the recovery of ambulation (Full *et al*, 2016; Mari *et al.*, 2019; Olby *et al.*, 2020). On the other hand, a more recent study done by Gouveia (2022), showed that the presence of SS was a negative prognostic factor as it unfavorably impacted ANNPE dogs' neurorehabilitation, so that it took more time for dogs affected by SS to regain ambulation (Gouveia *et al.*, 2022c), which is similar to what has been reported in human medicine (Christensen *et al*, 1990; Ko *et al.*, 1999; McBride, 2021).

Ko (2018) reported a direct correlation between the severity of the spinal cord transection, the profoundness of SS and its negative prognosis (Ko, 2018). This falls in line with what was concluded by Atkinson & Atkinson (1996), who stated that for a SCI with the same degree, the presence of SS causes a faster progression of injury and a worse prognosis (Atkinson & Atkinson, 1996).

1.4. Intensive neurorehabilitation

Physical neurorehabilitation is implemented in both veterinary and human medicine with the aim of aiding the recovery of ambulation status or improvement of the locomotion ability (Grasso *et al.*, 2004; Martins, 2015; Hodgson *et al.*, 2017) after injuries that affect the spinal cord. Furthermore, early introduction was demonstrated to be safe and well tolerated by dogs (Zidan *et al.*, 2018).

Neurorehabilitation includes multiple modalities that have the main goal of stimulating neuroplasticity and neuromodulation of the lesioned spinal cord either by strengthening existing synapses or creating new circuits (Grasso *et al.*, 2004; Côté *et al*, 2018; Martins *et al.*, 2021b). However, the plasticity promoted by neurorehabilitation is not necessarily always beneficial (Martins, 2015; Nielson *et al.*, 2016). Unwanted sprouting may occur leading to maladaptive sensory functions causing neuropathic

pain or maladaptive motor functions that will cause spasticity, thus caution must be taken when implementing these therapeutic modalities (Nielson *et al.*, 2016).

1.4.1. Locomotor training

Locomotor training is a repetition-based exercise aimed to promote, not only postural standing abilities (Gouveia *et al.*, 2022a), but also relearning stepping movements, and improving gait quality (Martins *et al.*, 2021a). LT stimulates central pattern generators (CPG) present in the lumbosacral segments of the spinal cord, which are responsible for generating a rhythmic stepping activity (Rossignol & Frigon, 2011), and relaying it to motoneurons (Grasso *et al.*, 2004). This translates to a coordinated firing and inhibition of flexor and extensor hindlimb muscle groups, as well as a left and right alternation, when there is a lack of supraspinal input and descending motor pathways (Martins *et al.*, 2021c). Locomotion recovery depends not only in the stimulation of CPGs, but also on the sensory input coming from the cutaneous afferents present on the foot. Although they do not interfere with the generation of the rhythmic stepping activity, they are important to modulate and readjust each step according to the environment (Rossignol & Frigon, 2011). In other words, they are responsible for foot placement and step progression (Côté *et al*, 2018).

Body weight supported treadmill training is reported to improve stepping capabilities with interlimb coordination (Wernig & Müller, 1992), by not only stimulating residual ascending and descending pathways, but also increasing the activity of the CPGs (Martins *et al.*, 2021a). Additionally, with progressive training, complete body weight bearing can be achieved by reducing the weight that is being supported (Wernig & Müller, 1992), which improves hindlimb muscle strength, promotes a better postural control and balance that is usually associated with better locomotor capabilities (Hubli & Dietz, 2013).

LT protocols will vary in speed, slope, and duration according to the aetiology (Gouveia *et al.*, 2022a). Animals can start with a bipedal training with the forelimbs on top of an immobile platform in order to acclimate to the treadmill, however quadrupedal training should be preferred, since it promotes the activation of residual descending circuits (Martins *et al.*, 2021d), in addition to promoting better coordination between forelimbs and hindlimbs, as they are controlled by different but codependent CPGs (Rossignol & Frigon, 2011; Hubli & Dietz, 2013). Forelimb and hindlimb coordination can also be enhanced by performing LT with a slope of 10-25º (Gouveia *et al.*, 2022a).

Underwater treadmill training (UWTT) is another form of LT. Due to the water's viscosity, weight bearing is provided, but it also creates some resistance that will aid in increasing muscle strength (Prydie & Hewitt, 2015; Barnicoat & Wills, 2016). Warm water should be used to stimulate the sensory afferents (Prydie & Hewitt, 2015), and similarly to land treadmill training, the speed, duration, slope, and water line will also vary according to the aetiology of each animal. It is important to note that the training should be

done under the supervision and monitorization of vital parameters, as well as being adapted to each animal depending on their cardiovascular and locomotor ability (Gouveia *et al.*, 2022a).

1.4.2. Kinesiotherapy exercises

Kinesiotherapy exercises are essential to promote muscle fibers regeneration, reconnection of the corticospinal pathways and enhancing neuroplasticity, ultimately promoting functional recovery (Frank & Roynard, 2018). These exercises focus on balance, proprioception, muscle strengthening, postural, gait stimulation (McCauley & Dyke, 2018) and range of motion (ROM). Depending on the dog's diagnosis and progression, exercises should be tailored to their needs and goals (Drum, 2010).

The goal for non-ambulatory animals is to regain functional tasks and avoid muscle atrophy, usually by performing standing positions with passive range of motion (PROM), which will stimulate muscles needed for balance, proprioception and strengthen hip and stifle extensors. In parallel, PROMs will maintain joint health (Drum, 2010) by maintaining synovial fluid production and distribution, enhancing blood and lymphatic flow and keeping soft tissue mobility (Millis & Levine, 2014). While performing static standing positions, it is important to maintain normal position in all four limbs to stimulate proprioceptive pathways and promote weight-bearing by shifting the animal's weight from side to side and front to back, slowly building resistance in all positions, and ultimately creating stability (Saunders, 2007; Millis & Levine, 2014). PROM consists of the joint movement, performed by a technician, without any voluntary muscle contraction, usually in a bicycle or walking-like pattern that can be done with the animal in lateral recumbency or in standing position (Millis & Levine, 2014). Toe pinching can also be used during these exercises, potentially to trigger the flexor withdrawal reflex, activate muscle contraction, and stimulate sensation (Drum, 2010). As the animal regains some strength, difficulty can be increased by utilizing uneven surfaces, such as cushions or balance boards (Saunders, 2007).

Animals that have recently regained ambulation may benefit with exercises that are more geared toward proprioception, gait training and muscle strengthening (Millis & Levine, 2014), through exercises such as sling-assisted walking combined with active assisted ROM or cavaletti obstacle rails (Drum, 2010). Active assisted ROM consists of the joint movement conducted by a technician with some assisted muscle contraction from the animal, which may be implemented during sling-assisted walking with the technician aiding the limb movement either on a land treadmill or on a water treadmill (Millis & Levine, 2014). When walking these patients, it is important to correct their feet position in cases of knuckling and prevent them from walking too fast as they may drag their hindlimbs (Drum, 2010). Cavaletti rails exercises increases significantly the flexion of the hip and stifle (Millis & Levine, 2014). It is also beneficial to stimulate the proprioceptive pathways, neuromuscular coordination (Drum, 2010), enhance balance and weight-bearing (McCauley & Dyke, 2018). Moreover, walking over cavaletti rails assists in attaining a normal limb extension during walking (Millis & Levine, 2014).

As the animal's neurologic state evolves and neuromuscular connection improves, active ROM and more complex exercises can be implemented to further enhance proprioception and joint stability (McCauley & Dyke, 2018). Examples of these exercises are walking on different surfaces such as gravel or sand, going up and down inclines, or walking in figure-eights (Drum, 2010). Active ROM occurs when the animal has voluntary control of muscle contraction and joint movement (Millis & Levine, 2014).

1.4.3. Electrical stimulation

Electrical stimulation is a non-invasive, neuromodulation therapy that is proved to aid in increasing muscle strength and tone, reducing pain, increasing ROM, enhancing function (Levine & Bockstahler, 2014) as well as motor relearning, that is, the ability to recover previously learned motor skills (Sheffler & Chae, 2007), by reproducing the muscle activation pattern that occurs during locomotion (Doucet *et al*, 2012). ES consists of applying a generated electrical current through leads and electrodes that are applied on surface of the skin, causing a depolarization of a motor nerve, which translates in muscular contraction (Levine & Bockstahler, 2014).

Functional electrical stimulation is one of the ES protocols, which is aimed to stimulate intact lower motor neurons near the motor point or through peripheral afferent nerves by using sequences of short bursts of electrical pulses (Martins *et al.*, 2021b), thus, potentially creating new neural connections (Martins *et al.*, 2021d). It promotes muscle strength and prevents further muscle atrophy and fatigue, by aiding in the conversion of type II (fast fatigable) muscle fibers back into type I (fatigue resistant) muscle fibers that have been lost after disuse (Gater *et al.*, 2011). FES also promotes increased ROM, circulation, and blood flow, decreases pain and spasticity (Doucet *et al*, 2012; Martins *et al.*, 2021a).

Electrodes for FES are applied along the desired peripheral nerve origin point and its motor point, after proper trichotomy, and while the patients are in standing position (Ragnarsson, 2008; Martins *et al.*, 2021a). Higher frequencies are usually used in order to produce a tetanic functional muscle contraction (Doucet *et al*, 2012), and although 20 Hz is enough to produce the tetanic muscle contraction, maximal muscle force contraction occurs between 60 to 100 Hz (Martins *et al.*, 2021b). However, one should keep in mind that higher frequencies causes increased muscle fatigue and may recruit pain fibers (Levine & Bockstahler, 2014). Therefore treatment should always be done under supervision and parameters should be adjusted in case the animal shows signs of pain or muscle weakness (Martins *et al.*, 2021a).

Transcutaneous electrical nerve stimulation (TENS) is another surface non-invasive modality used mainly for pain management by stimulating large mechano-sensitive afferent nerve (A-β) fibers present on the skin, consequently, overriding pain impulses transmitted by small cutaneous (A-δ and C) fibers (Doucet *et al*, 2012; Levine & Bockstahler, 2014; Sivaramakrishnan *et al*, 2018). This is done by using low frequencies, which will stimulate the sensory fibers without activating motor neurons (Doucet *et*

al, 2012). Besides pain management, TENS also showed that it temporarily provides non-spastic effects when electrodes were placed below the injury site with frequencies between 50-100 Hz (Martins *et al.*, 2021b). This anti-spastic effect is hypothesized to happen by synaptic neuromodulation and reorganization caused by the stimulation of large afferents, presynaptic inhibition (Levin & Hui-Chan, 1992), and reciprocal inhibition (Crone *et al.*, 1994). In human medicine, these spasticity suppression effects were reported to last up to four hours after application, both with FES and TENS, although the duration effects are proportional to the stimulation time (Sivaramakrishnan *et al*, 2018).

Evidence in human neurologic rehabilitation suggests that using the aforementioned therapy, in an isolated manner will not improve voluntary strength (Glinsky *et al*, 2007; Harvey, 2016); however, the implementation of a multimodal therapy, that is, a combination of ES, LT and kinesiotherapy exercises supports a faster and improved recovery in people after a neurologic injury, therefore this type of therapy should be provided to attain optimum results (Ragnarsson, 2008; Frank & Roynard, 2018).

1.4.4. Laser therapy

The concept behind laser usage is photobiomodulation, which consists of the use of a particular wavelength on the body causing cells to react to this light. It was reported to have a myriad of benefits. When combined with nerve transplantation, it was reported to decrease glial scar formation, promote axonal sprouting, increase electrophysiologic activity of a damaged peripheral nerve (Rochkind & Ouaknine, 1992) and enhance its repair (Gigo-Benato *et al* , 2005). It is also reported to have anti-inflammatory effects and being associated with decreased ambulation recovery time (Draper *et al.*, 2012; Veronez *et al.*, 2017). Another benefit that laser therapy provides is the relief from orthopedic and neuropathic pain (Gigo-Benato *et al*, 2005), which is thought to occur due to the increase in production of endogenous opiates, inhibition of the action potential of peripheral nerve nociceptors (Cotler *et al.*, 2015), suppression of pain-related neurotransmitters (Millis & Saunders, 2015), and reduction of nociceptor activity at the spinal level (Niebaum *et al*, 2018).

The type of laser used in rehabilitation is also called low light laser therapy (LLLT), which is a monochromatic, coherent, and collimated light, meaning that the light emitted comes in only one wavelength; photons travel all in the same phase and direction; and there is minimal deflection, allowing the laser beam to be focused on one specific part of the body and penetrate the skin without heating or damaging it. However, the laser light only has therapeutic effects if it gets absorbed by chromophores, otherwise it will not affect the tissues (Rochkind & Ouaknine, 1992; Millis & Saunders, 2015). The light wavelength that is usually used is in the red and infra-red regions (Cotler *et al.*, 2015) and it determines how deep it will penetrate the tissues. For example, 600–700 nm is ideal for treating superficial tissues, such as wounds and burns, as it promotes fibroblasts, collagen production and angiogenesis, hence

increasing the wound healing rate (Niebaum *et al*, 2018), and 750-1500 nm is preferred for deeper tissues, such as intra-articular structures, ligaments, and joint capsules (Millis & Saunders, 2015).

Laser therapy in the rehabilitation setting is applied using a hand-held laser probe perpendicularly to the skin and usually in contact with it, in order to minimize reflection and beam divergence. However, the *no contact* technique can be used when treating open wounds. The hair in the treating area must be clipped and, in cases where the dog's skin is darker, treatment dose may be increased by 25% (Millis & Saunders, 2015). The probe should be moved in a grid-like manner or fanning movement. Also, more frequent movements are required when treating darker skinned dogs to avoid overheating the skin. For deeper tissues treatment, a *point-to-point* technique can be employed, by holding the probe in one spot for a certain period of time and then moving to another, allowing a deeper penetration of the beam (Niebaum *et al*, 2018). Finally, any topical medication should be removed from the skin and the technician carrying this therapy, as well as the dog, should wear adequate eye wear to avoid retina damage (Millis & Saunders, 2015; Niebaum *et al*, 2018).

Laser is commonly used in a multimodal approach as the first phase of the treatment session, given that its main goal is to reduce pain and inflammation, so that following therapies such as ROM or kinesiotherapy exercises can be done to stimulate neuroplasticity and enhance muscle strength (Gross, 2014).

1.5. Spinal shock scale and its modifications

The spinal shock scale (SSS) is a numeric punctuation scale ranging from zero to seven, that evaluates the tonus and spinal reflexes of the hindlimbs, tonus and perineal reflex, as well as the cutaneous trunci reflex (Table 2). That being said, the prognosis becomes more guarded when the score is higher than four and a patient with a score of seven is considered to have profound signs of SS (Gouveia *et al.*, 2022c). Knowing that the *rectus abdomininis* muscle, along with other abdominal muscles, are important for the animal's correct posture, core stability, as well as provide support for their locomotion (Fischer *et al*, 2013), the author believes that the SSS should have a modification with this muscle's tone included.

Table 2: Spinal Shock Scale (Source: Gouveia et al, 2022c)

Reduced hindlimb reflexes	Withdrawal reflex	(+1)
	Patellar reflex	(+1)
	Cranial tibial reflex	(+1)
Reduced hindlimb tone		(+1)
Reduced perineal reflex		(+1)
Reduced perineal tone		(+1)
Absent/Abnormal cutaneous *trunci* cut-off		(+1)
TOTAL SCORE		

If total score ≥4: reserved prognosis; if total score is 7: profound signs of spinal shock.

1.6. Scale validity

Scales have been used widely, yet as science progresses and study fields become more specific, there has been a rising need for developing new scales (Boateng *et al.*, 2018). As the scale is being developed, it needs to go through a scale evaluation to test its reliability and validity. Scale validity is assessed by determining if the scale truly measures what it proposes and was developed to measure. This can be done with multiple techniques, the most common ways being content validity, criterion validity and construct validity (Rubio *et al.*, 2003).

Scale reliability evaluates its consistency over time and by different users, in other words, it evaluates if the same scale used in different times but under the same conditions will provide the same results (Boateng *et al.*, 2018). It should be noted that a scale can be valid but not reliable as well as reliable but not valid. That is why these two properties should be tested and satisfied to ensure the scale can the used with the purpose it was designed for (Raykov & Marcoulides, 2011).

1.6.1. Content and face validity

Content validity refers to the degree in which the components of the scale are relevant to what is being measured. Face validity evaluates the scale's ambiguity and appropriateness of each item.

Content and face validity should be evaluated by a panel of experts, usually around five to seven experts, that are independent to the ones involved in the scale's development. These experts then judge whether each item is adequate to the study domain (Raykov & Marcoulides, 2011). According to the

experts' feedback, the scale should then be adjusted before the final scale is put out to be tested by the target population (Boateng *et al.*, 2018; Tsai *et al*, 2019).

For Rodrigues (2017) validation of a new tool to measure preferences to exercise in people with osteoporosis, in order to evaluate the content and face validity, all experts were assigned to rate each item's relevancy, essentiality, and clarity. Regarding relevancy, items were rated with a value of one to four, one being "not relevant", two being "somewhat relevant", three being "quite relevant" and four being "very relevant", their essentiality with a scale of one to three: one being "not essential", two being "useful, but not essential" and three being "very essential", as well as their clarity on a scale of one to three, with one being "not clear", two being "item needs revision" and three "quite clear". Content validity was then quantified by using the content validity index (CVI) and the content validity ratio (CVR) (Rodrigues *et al.*, 2017).

CVI is calculated by adding up all the experts that rated an item as "very relevant" and dividing by the total number of experts. It is recommended that the CVI should be higher than 0.78. In case it is rated between 0.70 to 0.79, the item should be reviewed and if it is below 0.70 the item should be eliminated from the scale (Rodrigues *et al.*, 2017; Rubio *et al.*, 2003). However, if the panel consists of less than five judges, the CVI should be no less than one, in other words, all experts should agree on the content validity of that item (Polit & Beck, 2006).

CVR is used to measure each item's essentiality and it is calculated using the following formula: $\frac{Ne-(\frac{N}{2})}{N/2}$, where Ne is the number of experts that rated the item with a three or "essential" and N is the total number of experts. It ranges between -1 and one and its interpretation is that the higher the result, the higher is the agreement between experts (Rodrigues *et al.*, 2017).

1.6.2. Criterion validity

Criterion validity evaluates the scale's score correlation to another gold standard scale (concurrent validity). Sessler (2002) used the visual analog scale (VAS) as a gold standard for the validation of the Richmond Agitation-Sedation Scale (RASS). The correlation between these two scales was calculated using the Spearman's correlation coefficient (Sessler *et al.*, 2002), which can range from -1 to one. When the coefficient is zero it means the two scales do not have a correlation with each other, the closer to -1 or one, the stronger the correlation between the two scales (Puth *et al*, 2014).

Occasionally, there may not be a gold standard or an applicable criterion to compare to, which is considered a limitation to this type of validity. When that happens, criterion validity can be excluded from the validation study (Boateng *et al.*, 2018).

Predictive validity is also part of the criterion validity, as it attempts to find a correlation between the use of the scale with a future status. This was done for the validation assessment of the Canadian Neurological Scale (CNS) by Côté (1989), in which they detected that the total initial CNS score could be a predictor of death, morbidity, and recovery for daily life activities (Côté *et al.*, 1989), as well as for the FOUR Score, which concluded that for every one point addition of the total score decreased the chances of poor outcome and decreased the chances of in-hospital mortality by approximately 20% (Wijdicks *et al.*, 2005).

1.6.3. Construct validity

Construct validity evaluates the level of efficiency of the scale and analyses if the results from the scale are compatible with what is known in theory. This can be done by comparing the scale to other known scales or instruments, and testing if they yield the same result (convergent validity) (Boateng *et al.*, 2018).

For the FOUR score, this validity was evaluated by comparing the scale to the Glasgow Coma Scale, revealing a high correlation between them (Wijdicks *et al.*, 2005). In parallel, for the RASS validity study, the scale was compared to several other scales, such as the Sedation Agitation Scale, the Ramsay sedation scale, as well as the Glasgow Coma Scale, showing a strong correlation with all of them. All these correlations were calculated through the Spearman correlation coefficient (Sessler *et al.*, 2002; Wijdicks *et al.*, 2005).

1.7. Scale reliability

Scale reliability, as stated above, evaluates how consistent the scale is, independently of time (test-retest reliability), user (inter-rater reliability) and across items (internal consistency).

1.7.1. Test-retest reliability or intra-rater reliability

Test-retest reliability assesses how consistent the participant's score is, after using the scale multiple times. It is generally calculated by using the Pearson correlation coefficient. From a medical point of view, it might not be possible to execute this test as it is needed enough time between each test to ensure the participant does not remember the previous score given. But, simultaneously, the patient status may not always remain the same, in order for the score to be the same (Wijdicks *et al.*, 2005).

1.7.2. Inter-rater reliability

The inter-rater reliability represents the level of agreement amongst the participants. Generally, when there are more than two raters, it is calculated using the intraclass correlation coefficient (ICC) or the Cohen's weighted κ (Fig. 6) (Sessler *et al.*, 2002; Wijdicks *et al.*, 2005; Tsai *et al*, 2019). For inter-rater reliability, it is recommended at least a 0.8 level of agreement (McHugh, 2012).

Value of Kappa	Level of Agreement
0–.20	None
.21–.39	Minimal
.40–.59	Weak
.60–.79	Moderate
.80–.90	Strong
Above.90	Almost Perfect

Fig. 6: Kappa statistics and its interpretation. (Source: McHugh, 2012)

1.7.3. Internal consistency reliability

Internal consistency evaluates the correlation between each item of the scale. The most popular method to calculate internal consistency is with Cronbach's α. It should be noted that high values of α does not necessarily mean high internal consistency, as it could imply that the scale is redundant; in parallel, a low α value could mean an insufficient number of items or low correlation between each item. Acceptable α values range from 0.70 to 0.90 but the interpretation of this value should be done critically (Tavakol & Dennick, 2011).

2. Objectives

As it was previously described, SS is a paradoxical phenomenon considering that it shows signs of a LMN lesion in a UMN lesion. Although it has a sudden presentation, it also is quick to resolve itself, sometimes taking as little as two days, according to various authors (Blauch, 1977; Little, 1986; Hodshon & Thomas, 2018). Therefore, the aim of this study was to verify the presence of signs of spinal shock in the first 72 hours through the applicability of the SSS to evaluate the paraplegic patients with DPP and no DPP.

3. Materials and methods

Data for this retrospective, blinded cohort study was collected at two different rehabilitation centers in Portugal, Centro de Reabilitação Animal da Arrábida (CRAA, Setúbal, Portugal) and Centro de Reabilitação e Regeneração Animal de Lisboa (CR^2AL, Lisbon, Portugal), from September of 2017 to March of 2023. This clinical study was performed after the approval of the Lusófona Faculty of Veterinary Medicine ethics committee (No. 12-2023) as well as with the written consent of the owners.

3.1. Population inclusion

In this study, 249 dogs were included regardless of their breed, age, sex, and weight but had to meet the criteria of having a diagnosis of acute or peracute T3-L3 myelopathy confirmed with CT or MRI. It could include aetiologies of non-compressive myelopathies (FCEM or ANNPE) or compressive myelopathies (IVDH Hansen type I and IVDH Hansen type II) and had to be admitted into the centers no more than 72 hours post injury, regardless of undergoing a surgical approach or not. Dogs that had confirmed diagnosis of any other SCI in L4-S3 or peripheral neuropathies were excluded from the study as were all cats. Dogs were presented with paraplegia with or without DPP, therefore classified according to the Open Field Scale (OFS) as OFS 0 (without DPP) or OFS 1 (with DPP) (Olby *et al.*, 2001) and showed signs of spinal shock.

3.2. Study design

All dogs were subjected to an initial neurorehabilitation examination by the same Certified Canine Rehabilitation Practitioner (CCRP) instructor and examiner and were videotaped using a Canon EOS Rebel T6 1300D camera (Taichung City, Taiwan).

The neurorehabilitation exam included assessment of mentation (alert, depressed, stupor, and coma), passive posture (kyphosis, lordosis, scoliosis, and Schiff-Sherrington), gait, and proprioception. Gait was evaluated by walking the animal in a straight line in a non-slippery surface while supporting the hindlimbs, as they were paraplegic. Proprioception was evaluated by placing the dorsal surface of the limb on the floor and observing how long it took for the animal to return it to its normal position. The presence of spinal hyperesthesia was evaluated by applying pressure with the fingers on both sides of the vertebral column and noting the degree of discomfort of the animal.

The spinal reflexes of the hindlimbs were evaluated using an 18 cm Buck hammer, with the animal in lateral recumbency and in a calm controlled environment. The reflexes include the patellar reflex to evaluate the integrity of femoral nerve and the L4-L6 segments of the spinal cord (Fig. 7), the tibial cranial reflex to evaluate the sciatic nerve (L7-S1), as well as the withdrawal flexor reflex. The patellar reflex was elicited by striking the patellar tendon briskly while keeping the limb slightly flexed, normally expecting a

reflex extension of the limb. Similarly, the tibial cranial reflex is evaluated by striking the insertion of the cranial tibial muscle while maintaining the limbed slightly flexed, which should cause a momentarily flexion of the hock. Lastly, the withdrawal flexor reflex is a more complex reflex since it involves sensory neurons, from either the sciatic nerve (if stimulus is applied to the fifth digit) or the femoral nerve (if it is applied to the first digit), the sciatic nerve, which is responsible for the stifle, hock and digit flexion and the femoral nerve, which innervates the hip flexor (Freeman & Ives, 2020). It is elicited by applying a noxious stimulus, such as pinching the interdigital skin with either the fingers or a Halstead mosquito forceps with a gradual pressure while maintaining the limb in extension, which should cause the animal to withdraw its limb away from the noxious stimulus.

Fig. 7: Evaluation of the patellar reflex using a Buck hammer (photo kindly provided by CRAA/HVA).

The thoracic spinal reflexes include the biceps reflex which evaluates the integrity of the musculocutaneous nerve and the C6-C7 segments of the spinal cord, it is elicited by tapping the insertion of the biceps brachii tendon, while keeping the elbow slightly extended; this should cause either a contraction of the muscle or a small flexion of the elbow; the triceps reflex is elicited by striking the triceps tendon while the elbow is slightly flexed, resulting in an extension of the elbow. The triceps reflex tests the radial nerve and the C7-T1 segments of the spinal cord. Lastly, the flexor withdrawal reflex tests the C6-T2 segments of the spinal cord and the axillary nerve responsible for the shoulder flexion, the musculocutaneous nerve that causes the elbow flexion, as well as, the median and ulnar nerves responsible for the flexion of the carpus and digits (Lahunta, 2009; Freeman & Ives, 2020). It is elicited

the same way it was described for the hindlimb. In SS dogs, these thoracic limb reflexes should also be tested and should all be present and normal.

The perineal reflex was evaluated by pinching or running a Halstead mosquito forceps along the skin of the perineum and observing if there was a contraction of the anus. This evaluates the pudendal nerve, sacral spinal nerves, and sacral spinal cord segments (Lahunta, 2009). The cutaneous trunci reflex was evaluated using a Halstead mosquito forceps, pinching the skin on either side of the spinous process of each vertebra, starting from the iliac crest up to T1 until there is a visible reflex contraction of the cutaneous trunci muscle.

Lastly, deep pain perception was evaluated by applying pressure in the medial and lateral digits of the limb being tested using a Halstead mosquito forceps. An appropriate response to this test should be vocalization, looking at the limb or attempting to bite and should not be confused with the flexor withdrawal reflex (Freeman & Ives, 2020).

With this neurologic exam, the animals were classified according to their neurologic state with the Open Field Score (OFS) (Olby *et al.*, 2001), SSS and the Functional Rehabilitation Scale for DPP (FNR-DPN). The SSS (Table 2) were scored by two CCRP veterinarians who were blinded to one another, so that inter-rater reliability was attained. In case there was more than 20% disagreement between the two raters, a third one would be brought in and review the video tapings.

3.3. Evaluation time-points

Each patient was evaluated according to the diagram shown in the Fig. 8. The SSS, OFS and FNRS-DPN was evaluated both at the time of admission (T0) and at the 72h mark (T12), while the SSS was performed every six hours until the 72h mark (T0-T12).

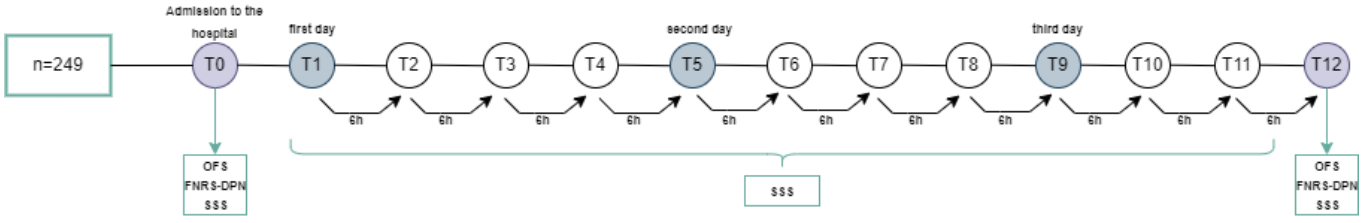

Fig. 8: Flowchart of all the evaluation time points during the first three days. T: Time; OFS: Open Field Score; FNRS-DPN: Functional Neurorehabilitation Scale for deep pain negative; SSS: Spinal Shock Scale.

3.4. Interventions

All dogs went through an intensive neurorehabilitation protocol that included FES modality, laser therapy and LT protocols, the latter included land treadmill training, underwater treadmill training and kinesiotherapy exercises. The protocols varied depending on aetiology and DPP, as shown in Table. 3.

Table 3: Intensive Neurorehabilitation Protocol

<table>
<tr><th></th><th colspan="2">Compressive Etiologies</th><th colspan="2">Non-Compressive Etiologies</th></tr>
<tr><td rowspan="2">Land Treadmill Training</td><td colspan="2" rowspan="2">• Six days/week
• 0.8 km/h →1.9 km/h
• Start with five to 20 min
• Four to six times/day → two times/day
• Increase to 30/40 min (2/3 times/day with 10% slope)</td><td>Without DPP</td><td>• Six days/week
• 1.5 km/h → 1.8 - 2.5 km/h
• Three to 10 min → 10 - 40 min
• Six to eight times/day → two to three times/day
• 2-5% slope → 10%</td></tr>
<tr><td>With DPP</td><td>• 6 days/week
• 1.8 km/h → 2 -2.5 km/h
• Three to 10 min → 10 - 40 min
• Four to six times/day → two to three times/day
• 2-5% slope → 10% slope</td></tr>
<tr><td rowspan="2">Underwater Treadmill Training</td><td colspan="2" rowspan="2">• Five days/week
• Two to seven days after admission
• One → 3.5 km/h
• Once a day
• Start with five min until one hour
• 26° C water temperature</td><td>Without DPP</td><td>• Five days/week
• 1.2 – 2 km/h → 2.8 - 4.5 km/h
• 10-20 min → 40 min
• Once a day
• No slope → 10% slope</td></tr>
<tr><td>With DPP</td><td>• Five days/week
• 1.2 - 2 km/h → 2 - 2.5 km/h
• Five to 10 min → 30 min
• Once a day
• No slope → 5%</td></tr>
<tr><td rowspan="2">Electrical Stimulation</td><td>Without DPP</td><td colspan="3">FES
Two to three times/day
40-60 Hz; 4-21 mA</td></tr>
<tr><td>With DPP</td><td colspan="3">FES
One to two times/day
40Hz; 4-16 mA</td></tr>
<tr><td rowspan="2">Kinesiotherapy Exercises</td><td colspan="2">Without DPP</td><td colspan="2" rowspan="2">• Starting with passive exercises, gradually progressing to passive active exercises and finally active exercises
• 10 min
• Two to four times/day</td></tr>
<tr><td colspan="2">With DPP</td></tr>
</table>

(min: minutes; DPP: Deep Pain Perception; FES: Functional Electrical Stimulation)

3.4.1. Functional electrical stimulation

Functional electrical stimulation (BTL® 4000 Smart, Famões, Portugal) is an electrical stimulation program performed ideally in the standing position, by placing the electrodes transcutaneously through the trajectory of the desired nerve.

In these cases, FES was performed on the hindlimbs specifically to stimulate the sciatic nerve. Therefore, after proper trichotomy, the cathode electrode was placed on the anatomic region for the sciatic nerve root (L7-S1) while the anode was placed near the motor point region of the flexor muscle. Water was sprayed in these areas to enhance conduction of the electrical current and electrodes were held in place by straps (Fig. 9).

Patients with DPP were stimulated one to two times a day with 40Hz and 4-16mA, whilst patients with no DPP were stimulated two to three times a day with 40-60Hz and 4-21mA. This is aimed to strengthen the contraction of the flexor muscles.

Fig. 9: Application of FES on the sciatic nerve (photo kindly provided by CRAA/HVA).

3.4.2. Land treadmill locomotor training

All animals were acclimatized to treadmill LT (Superior Fit Fur Life Treadmill®, Fernhurst, Surrey, UK) by starting in a bipedal training, which was performed by placing the forelimbs in a fixed platform above the treadmill belt while the rehabilitator executed passive bicycle movements on the hindlimbs and, eventually, evolved into the preferred quadrupedal training. During the first stage, body weight support was used since the patients were paraplegic. In addition, due to the hypotonicity of the flexor muscles, bicycle movements were performed with limb stretching in order to stimulate the Ia intrafusal fibers, which are sensory fibers responsible for proprioception and muscle tone; pressure was applied to the digits against the treadmill belt to stimulate cutaneous afferent receptors stimulating the CPGs responsible for generating the rhythmic stepping movement (Fig. 10). Once the animal regained some movement, the rehabilitator started stimulating just the tip of the tail or the perineum which would further encourage stimulation of the intrinsic circuits and the CPGs.

Fig. 10: Land treadmill training (photo kindly provided by CRAA/HVA).

Depending on the animal's aetiology the speed programmed on the treadmill differed. In the initial phase, post-surgical animals (IVDH) started with a lower speed (0.8 km/h), each session was three to five minutes long, which were performed four to six times a day, aiming for 30-40 minutes twice a day with 1.9 km/h speed and 10% slope. Non-surgical animals (ANNPE and FCEM) could start with higher speeds (1.5 km/h), each session lasting for three to 10 minutes, six to eight times a day, with a slope of 2-5%, aiming for 10 to 40 minutes twice or three times a day, with a speed of 1.8-2.5 km/h and a slope of 10%. Each patient was closely monitored while performing the LT, as well as any posture deviation, weight bearing, and balance was corrected accordingly. Additionally, the session was halted if the animal showed any sign of discomfort or fatigue.

3.4.3. Underwater treadmill locomotor training

Underwater treadmill training (Hidro Physio®, Broseley, UK) started as soon as possible for non-surgical patients while post-surgical patients started two to seven days after hospital admission with special caution being taken to avoid getting the suture wet and, consequently, reducing risk of infection. Water temperature was kept at around 26ºC to allow proper muscle relaxation and increase blood flow, while also preventing respiratory complications associated with colder temperatures. Similarly to the land treadmill training, the rehabilitator's role during the first stages is fundamental to help execute the bicycle movements inside water, which is later on replaced by just perineal and tail stimulation to encourage independent hindlimb movement (Fig. 11).

Fig. 11: Underwater treadmill training (photo kindly provided by CRAA/HVA).

IVDH patients started with a speed of one km/h for five minutes per session, gradually increasing speed to 3.5 km/h and aiming for one hour a session, whereas ANNPE and FCEM patients with DPP started with no slope, a speed of 1.2 to 2 km/h for five to 10 minutes, progressively increasing it to 2-2.5 km/h for 30 minutes and 5% slope. Patients with no DPP started also with no slope, with a speed of 1.2-2 km/h for three to 10 minutes, progressing to 2.8-4.5 km/h for 40 minutes and 5% slope.

As it happened in the land treadmill training, patients were closely monitored while performing the exercise and the session was interrupted if they started showing signs of discomfort or fatigue. After the exercise, they were properly dried in order to avoid secondary complications.

3.4.4. Kinesiotherapy exercises

Kinesiotherapy exercises were performed by all animals in this study, though difficulty levels varied according to their neurological status. In the first stage of the rehabilitation protocol, passive exercises, such as bicycle movements and flexor exercises were performed in a standing position under a rough surface to stimulate interdigital cutaneous receptors (Fig. 12). Depending on the animal's size, two or

more rehabilitators might be needed in this first stage to maintain the stability of the animal's vertebral column.

As their neurological status evolved and regained some muscle contraction, the exercises advanced into active assisted ones, such as body weight supported gait stimulation in alternated floor surfaces and cavaletti rail exercises that were done to enhance the animal's proprioception and balance. Once the patients regained muscle control and range of motion and, thus, were able to perform active exercises, that is, the previous exercises with no body weight support, more active exercises were implemented, which included up-down ramps, vertical pole exercises and balance board postural standing. Each session was 10 minutes long and was performed two to four times a day.

Fig. 12: Kinesiotherapy exercise: Bicycle movements on a rough surface (photo kindly provided by CRAA/HVA).

3.4.5. Laser therapy

Laser therapy was performed with a class IV laser (Companion Therapy Laser— LiteCure®, New Castle, DE, USA) for pain control to all patients that showed evidence of having painful muscle trigger points. The handheld probe was put perpendicular and in contact with the skin that was previously trichotomized and moved in a grid like pattern over the area of treatment (Fig. 13). Parameters were set to 12 J/cm^2, 2.5Hz, duty cycle 88% and 3-minutes pulsed mode. In addition, laser therapy was initiated for patients that had developed pressure ulcers with the aim of shortening the healing time. Parameters were set to 2W, 30 seconds with 20Hz, 500Hz, 5000Hz and 10000Hz, in this order. The hand probe was also moved in a grid like pattern, perpendicular to the skin but without contact with the ulcer to avoid wound infection. Appropriate eyewear was worn by the rehabilitator who was performing the therapy and care was taken to avoid pointing the laser to the eyes of the patient to avoid retina damage.

Fig. 13: Laser therapy for pain control (Photo kindly provided by CRAA/HVA).

3.5. Data collection

Data was collected from all dogs (n=249) including age, weight, sex, breed, chondrodystrophy, aetiology, presence of deep pain perception, time until admission, development of myelomalacia, and clinical complications. Clinical complications could include aspects such as fever, diarrhea, pneumonia, and/or nasal discharge.

Continuous quantitative variables such as age and weight were categorized into “<7 years old” and “≥7 years old”; “<15kg” and “≥15 kg”, respectively. Categorical binominal variables were also categorized, namely sex into “female” or “male”, breed into “pure breed” or “mixed breed”, chondrodystrophy - “chondrodystrophic breeds” or “non-chondrodystrophic breeds”, aetiology “compressive” or “non-compressive”, presence of deep pain “present” or “absent”, and clinical complications “present” or “absent”. Lastly, categorical nominal variables included time until admission that was categorized in “≤24h”, “24-48h” or “48-72h” and development of myelomalacia that was categorized in “absent”, “descending”, or “progressive”.

Categorical ordinal variables such as the scores from the SSS, OFS and FNR-DPN were also reported. SSS scores were categorized in “<4” and “≥4” and the OFS scores were categorized in “0” and “1”.

3.6. Statistical Analysis

All data was documented in Microsoft Office Excel 365 (Microsoft, EUA) and handled with Statistical Package for the Social Sciences (SPSS) 25 software (IBM, EUA). Arithmetic means, standard error of mean (SEM) and standard deviation (SD) were done for all parametric data, while median and range were calculated for continuous variables of age and weight (non-parametric data). Kolmogorov-Smirnov normality test was calculated for n>50 and descriptive statistics with frequency analysis were

performed for nominal categorical variables. In order to establish relevant levels of significance, Chi-square test was calculated (p-value ≤ 0.05), and the estimated marginal means with interaction plots were determined for the SSS for all six-hour interval time points.

The study population stablished a type I error (α) of 0.01. This error calculates the probability of a false positive conclusion and generally investigators aim for less than 5% chance of getting a false positive conclusion, therefore this type of error is set to 0.05. Type II error (β) calculates the probability of a false negative conclusion, and the power (1-β) represents the probability of avoiding a false negative conclusion which is normally set to be 0.80 (Schulz & Grimes, 2005). In this study, the power was calculated to be approximately 0.90.

4. Results

In this study, 249 dogs (n=249) fit the inclusion criteria and so were included in this study. Table 4 presents the results regarding the characterization of the population.

Table 4: Characterization of the total study population

n=249	
Age	<7 yo: 86.3% (215/249)
	≥7 yo: 13.7% (34/249)
	Mean: 4.65 yo
Weight	<15kg: 85.1% (212/249)
	≥15kg: 14.9% (37/249)
	Mean: 10.59kg
Sex	Male: 61.8% (154/249)
	Female: 38.2% (95/249)
Breed	Breed: 79.1% (197/249)
	Mixed breed: 20.9% (52/249)
Condrodystrophy	Condrodystrophic: 69.9% (174/249)
	Non-condrodystrophic: 30.1% (75/249)
Aetiology	Compressive: 86.7% (216/249)
	Non-compressive: 13.3% (33/249)
Deep Pain Perception	with DPP: 62.7% (156/249)
	without DPP: 37.3% (93/249)
Time until admission	≤24h: 12.9% (32/249)
	24-48h: 58.6% (146/249)
	48-72h: 28.5% (71/249)
Clinical complications	Present: 91.2% (227/249)
	Absent: 8.8% (22/249)
Myelomalacia	Absent: 92.0% (229/249)
	Descendent: 5.2% (13/249)
	Progressive: 2.8% (7/249)

(yo: years old; DPP: Deep pain perception)

4.1. Age and weight

The patients' age ranged from one year to 12 years and, as described in Table 5, it had a mean age 4.65 years, median and mode of five, variance of 4.50 and standard deviation of 2.12. As it was referred previously, the data were categorized in "<7 years old" which revealed to be the predominant category with 86.3% (215/249), while patients with "≥7 years old" represented 13.7% (34/249) of the total population.

As for the patients' weight it ranged from two to 45 kg. The mean was calculated to be 10.59, median of nine, mode of seven, variance of 49.928 and standard deviation of 7.066 (Table 5). Patients with "<15 kg" rounded up to be most of the population with 85.1% (212/249) while patients with "≥15kg"

represented 14.9% (37/249). As the sample size was rather large (n>50), the Kolmogorov-Smirnov normality test was performed instead of the Shapiro-Wilk normality test (Mishra *et al.*, 2019) revealing that for these two continuous variables showed lack of normality (p≤0.001).

Table 5: Descriptive analysis of the total population's (n=249) age and weight

	Total population (n=249)	
	Age (years)	**Weight (Kg)**
Mean	4.65	10.59
Standard error of mean (SEM)	0.134	0.448
Median	5	9
Mode	5	7
Standard deviation (SD)	2.12	7.066
Variance	4.494	49.928
Minimum	1	2
Maximum	12	45
Kolmogorov-Smirnov normality test	<0.001	<0.001

(SEM: Standard error of mean; SD: Standard deviation)

4.2. Sex

According to the data presented in this study, the majority (61.8%) of the patients were “male” (154/249) and 38.2% were “female” (95/249) (Fig. 14).

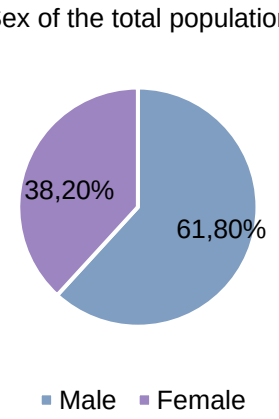

Fig. 14: Graphic representation of the sex percentage of the total study population.

4.3. Breeds

The study included 79.1% dogs of pure breed (197/249) and 20.8% of mixed breed (52/249). These included French Bulldog (n=71), Dachshund (n=33), Pekingese (n=16), Yorkshire Terrier (n=16), Poodle (n=8), Beagle (n=7), Labrador (n=6), Jack Russell Terrier (n=5), Shih Tzu (n=5), Cocker Spaniel (n=4), Maltese Bichon (n=3), English Bulldog (n=3), Pinscher (n=3), Pug (n=3), Basset Hound (n=2), Portuguese Podengo (n=2), Boxer (n=1), Chihuahua (n=1), Dogue de Bordeaux (n=1), German Shepperd (n=1), Siberian Husky (n=1), Pitbull (n=1), Portuguese water dog (n=1), Rottweiler (n=1), Spitz (n=1), and West Highland White Terrier (n=1), as represented in Fig. 15. As a result, 67.9% (169/249) were chondrodystrophic breeds and 32.1% (80/249) were non-chondrodystrophic breeds.

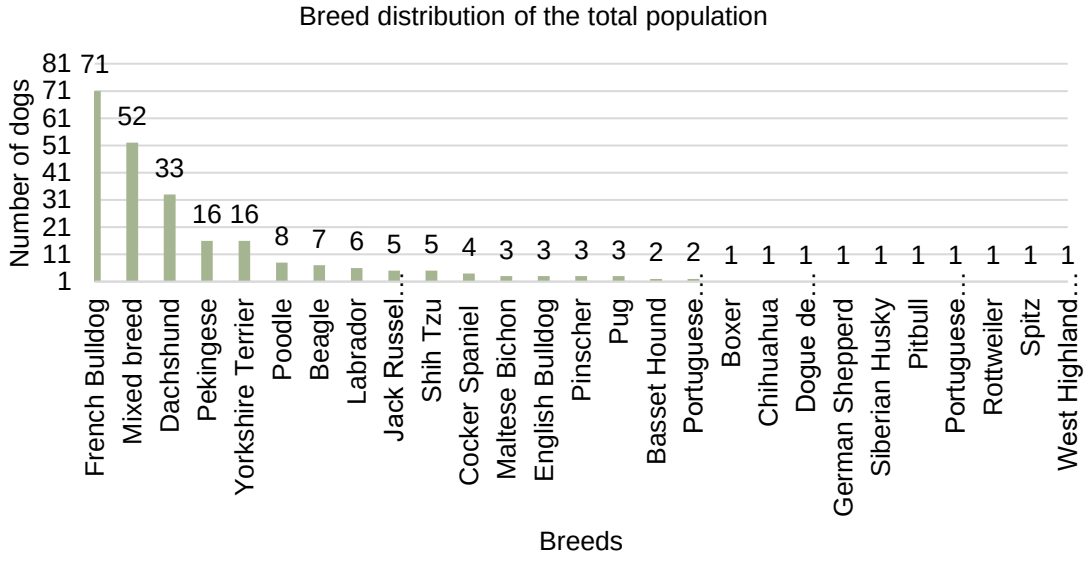

Fig. 15: Graphic representation of the breed distribution of the total study population

4.4. Aetiology

When it comes to aetiology (Fig. 16), 86.7% of the total population (216/249) came with a “compressive” myelopathy diagnosis, of which 214 were IVDH type I and two were IVDH type II, while 13.3% (33/249) had a “non-compressive” myelopathy diagnosis, of which 22 were ANNPE and 11 were FCEM.

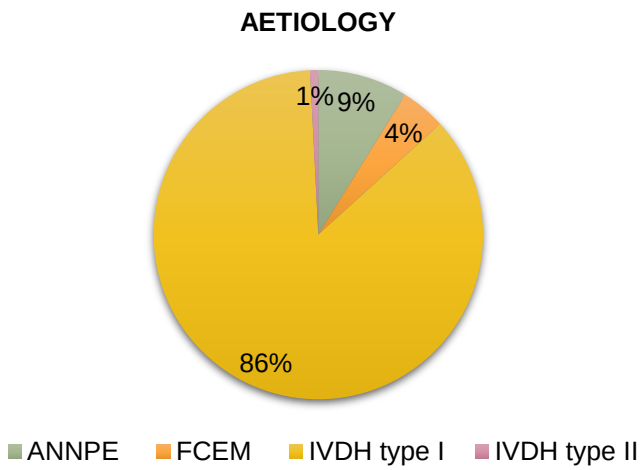

Fig. 16 Graphic representation of the aetiologies that affected the total population. (ANNPE: Acute Non-Compressive Nucleus Pulposus Extrusion, FCEM: Fibrocartilaginous Embolic Myelopathy, IVDH: Intervertebral Disc Hernia).

4.5. Deep pain perception

At the time of presentation, 62.7% (156/249) of the dogs had DPP, therefore had an OFS score of "1", whereas 37.3% (93/249) of the dogs did not have DPP, which meant that their OFS score was "0" (Fig. 17) and FNRS-DPN score was ≤6. All dogs that had DPP were able to regain ambulation, however only 9.7% (9/93) of the negative DPP group at T0 were able to regain DPP (Table 6) and the ambulation status.

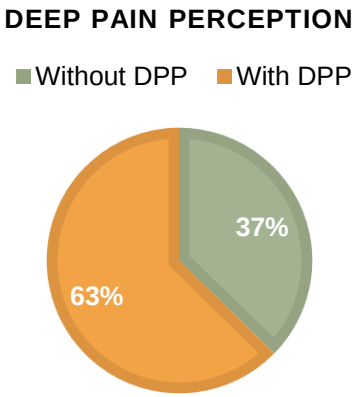

Fig. 17: Graphic representation of the population's deep pain perception at the time of admission. (DPP: Deep pain perception).

Table 6: Characterization of the nine dogs that were able to regain deep pain perception.

Identification	Etiology	Time until admission (hours)	SSS (T0)	Recovery of SS (hours)
1	IVDH type I	48-72	1	12
2	IVDH type I	48-72	1	18
3	IVDH type I	24-48	2	12
4	ANNPE	24-48	1	6
5	IVDH type I	48-72	1	12
6	IVDH type I	48-72	1	24
7	ANNPE	≤24	3	12
8	FCEM	≤24	3	6
9	ANNPE	≤24	3	6

(IVDH: Intervertebral Disc Hernia; ANNPE: Acute non-compressive nucleus pulposus extrusion; FCEM: Fibrocartilaginous Embolic Myelopathy; SSS: Spinal Shock Scale)

4.6. Time until admission

Regarding time until admission, 58.6% (146/249) of the patients were admitted during the "24-48h" post-injury period, 28.5% (71/249) in the "48-72h" period and 12.9% (32/249) gave entry before "≤24h" (Fig. 18).

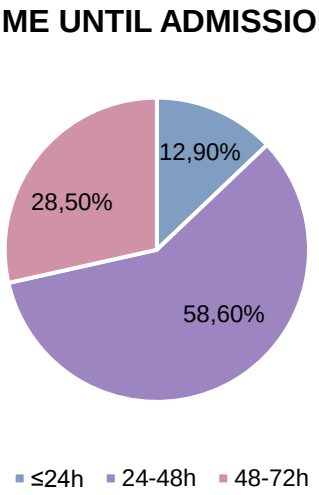

Fig. 18: Graphic representation of the time until admission of the total study population.

4.7. Clinical complications

Regarding clinical complications, they were present in 91.2% (227/249) of the patients while 8.8% (22/249) did not develop any clinical complications (Fig.19).

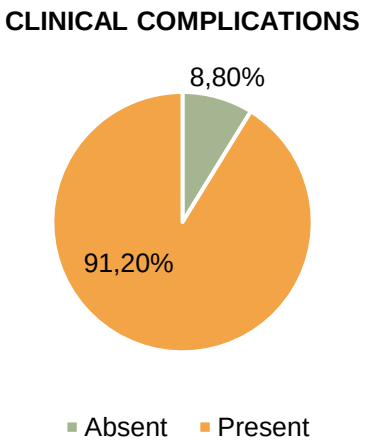

Fig. 19: Graphic representation of the presence of clinical complications in the total of the population.

4.8. Myelomalacia

In the present study, 8% (20/249) of the total population developed myelomalacia, of which 13 dogs were “descending” myelomalacia and seven were “progressive” myelomalacia (Fig. 20). None of these patients were able to regain ambulation and all dogs with progressive myelomalacia were euthanized.

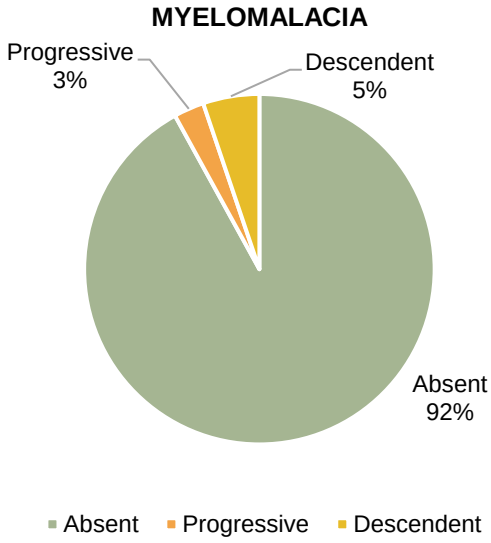

Fig. 20: Graphic representation of the development of myelomalacia in the total population.

4.9. Spinal Shock Scale

The SSS scores ranged from one to seven and, as characterized in Table 7, had a mean of 4.12, standard error of mean of 0.098, median of four, mode of three, standard deviation of 1.551, and variance of 2.405.

Table 7: Characterization of the SSS scores from the total population

	Scores of the SSS
Mean	4.12
Standard error of mean (SEM)	0.098
Median	4
Mode	3
Standard deviation (SD)	1.551
Variance	2.405
Minimum	1
Maximum	7

When it comes to the SSS inter-rater reliability, there was a 18% disagreement between the two raters with a total of 6 474 observations.

As mentioned previously, the SSS scores were divided into "<4" and "≥4" categories, of which 57% (142/249) had a score of "≥4" and 43% (107/249) had a score of "<4" at T0.

Regarding age, 116 patients with "<7 years old" had a SSS "≥4" score at T0 showing a significance of x^2 (1, n=249) = 6.074, p = 0.014), in contrast, no significance was found considering the weight of the patients. Likewise, both sexes revealed a higher prevalence of SSS "≥4" scores (55 females and 87 males).

Considering chondrodystrophy, both chondrodystrophic and non-chondrodystrophic breeds showed similar results regarding having a SSS score "≥4" (55.7% for chondrodystrophic breeds and 60% for non-chondrodystrophic breeds), however when it comes to dogs of pure breed, out of the 112 that presented with SSS "≥4" scores at T0, 97 patients were of a chondrodystrophic breeds. Additionally, of the 216 patients with compressive aetiology, 118 presented with SSS "≥4" scores at T0 showing a significance of x^2 (1, n=249) = 3.826, p=0.05.

When it comes to DPP, out of the 93 patients that did not have DPP, 84 had a SSS "≥4" score while only 58 of the patients that had DPP (n=156) presented with a SSS "≥4" score (x^2 (1, n=249) = 67.147, p≤0.001).

There was also shown strong statistical significance toward the SSS score at T0 and time until admission ($x^2(2, n=249) = 33,590$ ($p \leq 0.001$). At “≤24h” and “24-48h” there was a predominant number of patients with SSS “≥4” scores (n=29 and n=90, respectively), whilst at “48-72h” there was more patients with SSS “<4” scores (n=48). Additionally, there was a marked decline in the SSS scores throughout these 72h (Fig. 21), especially in the first six hours.

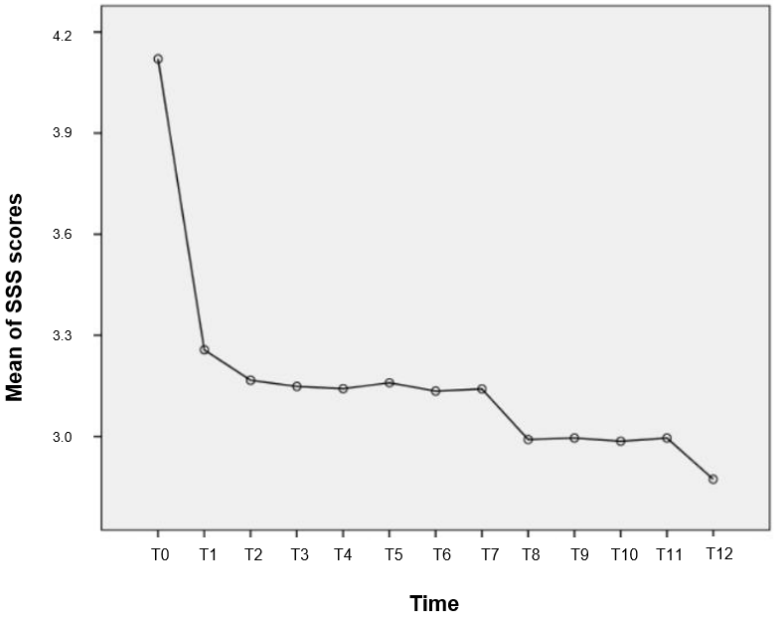

Fig. 21: Interaction plot showing SSS score means for the first 72h every 6h. X-axis: Time points (T0: admission day; T1:6h; T2: 12h; T3: 18h; T4:24h; T5: 30h; T6: 36h; T7: 42h; T8: 48h; T9: 54h; T10: 60h; T11: 66; T12: 72h); Y-axis: Spinal shock score.

Figures 22 through 25 reflects the evolution of the SSS score throughout time according to aetiology (Fig. 22), DPP (Fig. 23), time until admission (Fig. 24) and SSS initial score (“<4” or “≥4”) (Fig. 25).

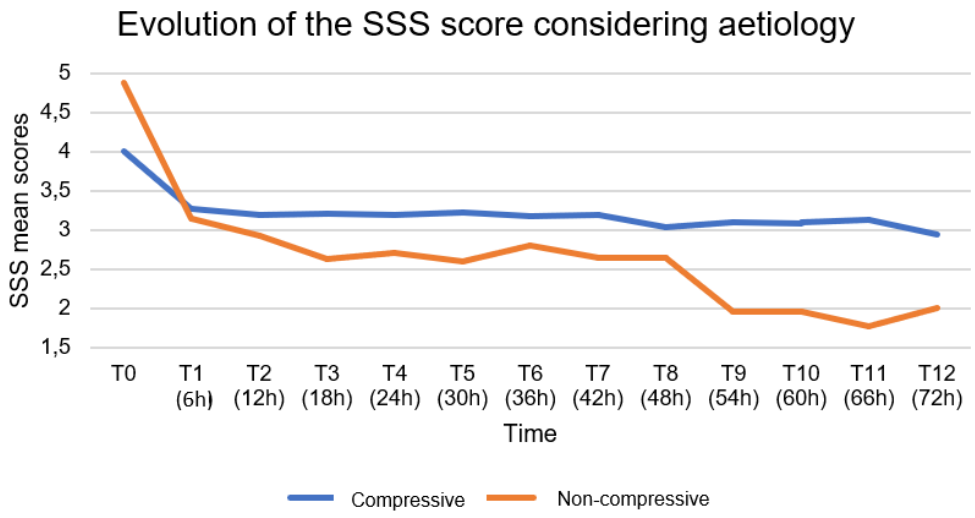

Fig. 22: Evolution of the SSS score considering aetiology (SSS: Spinal Shock Scale; T0: Day of admission)

According to Fig. 22, non-compressive aetiologies presented at admission with a higher SSS score yet they also showed a more drastic evolution, reaching a mean SSS score of two at the 72h time point while compressive aetiologies present at admission with a lower SSS score but also reveals a more plateaued evolution.

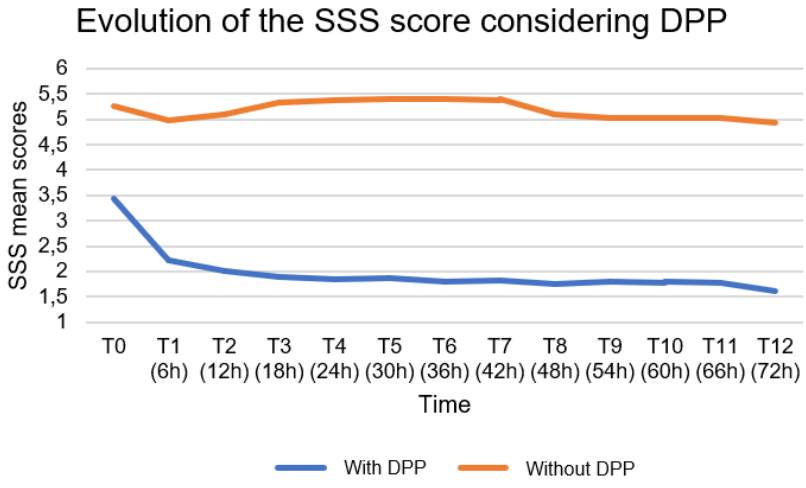

Fig. 23: Evolution of the SSS score considering DPP (SSS: Spinal Shock Scale; T0: Day of admission; DPP: Deep pain perception)

As shown in Fig. 23, patients with DPP had a lower SSS score at admission compared to the patients without DPP, the former group also revealed a more pronounced recovery compared to patients without DPP which showed to have a less drastic recovery.

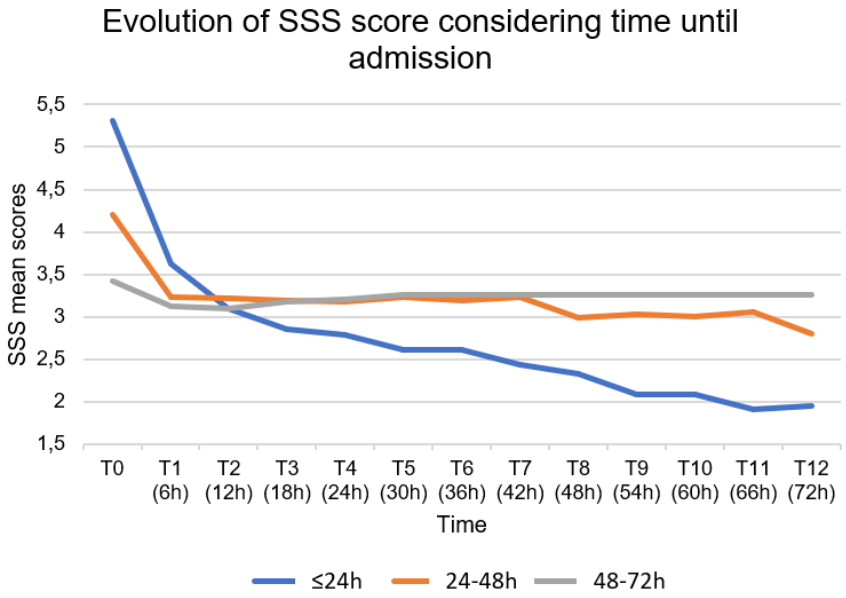

Fig. 24: Evolution of SSS score considering time until admission (SSS: Spinal Shock Scale; T0: Day of admission)

Regardless of time until admission (Fig. 24), all groups displayed a reduction of SSS score from admission time (T0) to T1, however patients that were admitted at “≤24h” presented a more drastic reduction, from a mean score of 5.3 at T0 to 1.9 at T12, in contrast, patients admitted at “48-72h” had a slower reduction, from a mean score of 3.4 at admission to a 3.2 score at T12.

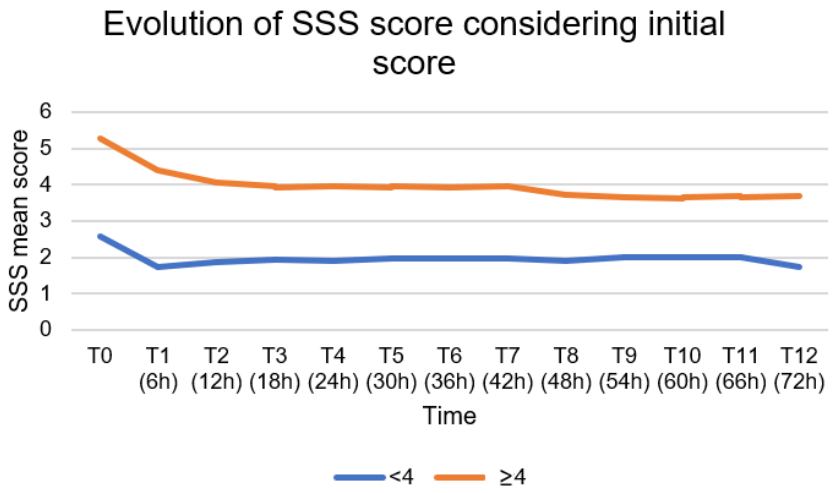

Fig. 25: Evolution of SSS score considering the initial score (SSS: Spinal Shock Scale; T0: Day of admission)

Considering the evolution of SSS with the initial SSS score (Fig. 25), both groups had a reduction from T0 to T1 but maintained a slower recovery until T12.

Table 8 reflects the patients that reached SSS score of 0 throughout the timestamps, and thus were removed from the rest of the study.

Table 8: Number of patients that reached SSS=0 throughout time

Time	Number of patients
T1	9
T2	4
T3	3
T4	5
T5	3
T6	2
T7	0
T8	6
T9	0
T10	2
T11	4
T12	4

(DPP: Deep pain perception; T1: 6h; T2: 12h; T3: 18h; T4: 24h; T5: 30h; T6: 36h; T7: 42h; T8: 48h; T9: 54h; T10: 60h; T11: 66h; T12: 72h)

All seven patients that showed signs of progressive myelomalacia were also presented with an initial SSS score of seven and did not show any signs of improvement. Consequently, they were euthanized in the first 72h.

5. Discussion

The aim of this study was to report the presence of SS signs during the first 72h after admission, with the applicability of the SSS in patients with and without DPP. The recovery of the pelvic reflexes is essential for the ultimate recovery of ambulation; therefore, neurorehabilitation protocols were implemented to aid achieving this goal. Neurorehabilitation aims to promote functional recovery by basing itself in the ability of the central nervous system to regenerate and reorganize, either by the modification of pre-existing synapses or as a result the sprouting of new axonal branches and dendrites (Raineteau & Schwab, 2001); and neuromodulation, which allows the regulation, through inhibition, stimulation or the modification of the activity of the nervous system (Krames *et al.*, 2009). This reorganization will allow not only the generation of movement through the recruitment and adjustment of the number of motor neurons but also stimulate cutaneous afferents that will affect the neurons excitatory net (Côté *et al*, 2018).

The present study revealed a predominance of patients with less than seven years old and less than 15kg. Similarly to the studies by Full *et al* (2016) and McBride *et al* (2022), this study showed a median age of five years old (Full *et al.*, 2016; McBride *et al.*, 2022), while the median weight of 10 kg was only compatible with the latter study. However, this study seems to deviate from McBride's *et al* (2022) when it comes to the association of SS with the patient's weight. While McBride *et al* (2022) concluded that lower weight patients were more likely to develop SS (McBride *et al.*, 2022), this study did not show any significance toward it.

Regarding the aetiology, this study is in agreement with what was reported by Hodshon & Thomas (2018) and McBride *et al* (2022), who state that the most common aetiology associated with spinal shock was the IVDH (Hodshon & Thomas, 2018; McBride *et al.*, 2022), however, it is not consistent with Full *et al* (2016) who report a higher prevalence of SS in dogs with FCEM (Full *et al*, 2016). The higher prevalence of IVDH may be justified by the fact that 86.7% (216/249) of the total population were diagnosed with a compressive myelopathy, of which 214 were IVDH type I, in addition to the fact that 67.9% (169/249) patients were of a chondrodystrophic breed, which are known to have a higher predisposition of developing IVDH type I due to the presence of an expressed retrogene (*FGF4*) in a chromosome 12 locus (Brisson, 2010; Fenn & Olby, 2020). IVDH type I is characterized as an extrusion of the NP into the vertebral canal due to a chondroid metaplasia, which compromises the ability of the IVD to sustain pressure, and it occurs most commonly between the ages of three and seven in chondrodystrophic breeds (Brisson, 2010; Coates, 2012), which is related to the predominant age of the study population discussed above.

The presence of deep pain perception is often a prognostic factor regarding the probability of the patient's likelihood to be able to regain independent ambulation (Jeffery *et al.*, 2016; Olby *et al.*, 2020; Martins *et al.*, 2021b). As a reflection, in this study, only the patients that had the presence of DPP at the time of admission and the ones that were able to regain it (9/93) managed to achieve ambulation. This prognostic factor was also confirmed by the fact that patients without DPP showed a higher SSS score at admission as well as a slower decrease in that score compared to patients with DPP, as shown in Fig. 17. Additionally, the literature reports that approximately 10-14% of the patients without DPP will develop progressive myelomalacia (Scott & McKee, 1999; Olby *et al.*, 2004; Aikawa *et al.*, 2012; Martins *et al.*, 2021e). However, this study presented a much lower percentage (3%), even when compared to the 4% reported by Jeffery *et al* (2020) after submitting patients to a decompressive durotomy to improve tissue perfusion (Jeffery *et al.*, 2020). Moreover, according to the reported results, the presence of SS does not seem to increase odds of developing myelomalacia.

More than 70% of the patients were admitted in the first 24h and 48h post-injury. That allowed for an early detection of SS signs and the subsequent recovery of some reflexes. This is characterized as the first and second phase of SS described by Ditunno (2004) in human patients (Ko *et al.*, 1999; Ditunno *et al.*, 2004). According to Ko *et al* (1999), the cremasteric and bulbocavernosus reflexes, in other words, polysynaptic cutaneous reflexes, are the first to reappear within the first three days in human patients followed by deep tendon reflexes (Ko *et al.*, 1999; Ditunno *et al.*, 2004). Likewise, in dogs affected by SS, the first reflex to reappear is the anal sphincter reflex, generally in the first 15 minutes post-injury, followed by the patellar reflex after 30 minutes to two hours (Blauch 1977 cit. by Smith & Jeffery, 2005).

In lower animals, the extrapyramidal tracts (rubrospinal, reticulospinal and vestibulospinal), which originate from the brainstem and travel in the ventral and lateral funiculi of the spinal cord, are thought to communicate with the motor neurons present in the pelvic limbs, as opposed to humans, which is the corticospinal tract. Consequently, the lack of their input reflects in the depression of the fusimotor tone that translates to loss of muscle tone and reflexes, likely contributing to the development and progression of SS (McBride, 2021).

Considering the above mentioned, the early implementation of a neurorehabilitation protocol is critical in profound cases of spinal shock as they are associated with a more guarded prognosis (Christensen *et al*, 1990; Ko, 2018). Additionally, the early application of neurorehabilitation protocols is linked to faster locomotor recovery while the introduction after longer periods leads to only occasional recoveries (Piira *et al.*, 2019; Yu *et al.*, 2019). These protocols aim to promote neuroplasticity through the sprouting of new circuits and strengthening synapses in spared descending motor axon tracts (Ditunno *et al.*, 2004; Grasso *et al.*, 2004; Côté *et al*, 2017; Martins *et al.*, 2021b), in order to stimulate the central pattern generators (CPGs) responsible for the rhythmic alternating stepping pattern (Ditunno *et al.*, 2004; Rossignol e Frigon, 2011; Martinez *et al.*, 2012). Therefore, it is important to detect signs of SS as well as classify it precisely

using the SSS, both at admission and all throughout treatment. With this study, it was observed that 57% (142/249) of the patients had a SSS ≥ 4, which meant that they were presented with a more guarded prognosis (Gouveia *et al.*, 2022c). Nonetheless, although the recovery in these patients can be more drawn out, as seen in Fig. 25, it was reported that it may not be an impediment to regaining ambulation (Full *et al*, 2016; Mari *et al.*, 2019; Olby *et al.*, 2020; Gouveia *et al.*, 2022c).

Patients that were admitted in the first 48h revealed higher SSS scores, but also more drastic decreases, as shown in Fig. 24, as expected with the natural progression of SS. Reflexes are lost below the injury site (Smith & Jeffery, 2005; Ko, 2018), immediately after the SCI, most likely due to the hyperpolarization of the α motor neurons, decreased activity of the γ-motor neurons and the increase of glycine, a inhibitory neurotransmitter (Schadt & Barnes, 1980; Simpson *et al,* 1996; Nacimiento & Noth, 1999; Dietz & Colombo, 2004; Ditunno *et al.*, 2004; Smith & Jeffery, 2005; Hodshon & Thomas, 2018; McBride, 2021). As new excitatory synapses grow, either from spared α-motor neurons or spinal interneurons, the depressed reflexes transit into an excitatory state, which may develop into spasticity (Ko *et al.*, 1999; Sheean, 2002; Ditunno *et al.*, 2004; Smith & Jeffery, 2005).

Fig. 21 presents the overall significant decrease of SSS marginal means in the first six hours with a relative plateau in the following hours. This can be explained by the slower recovery of the withdrawal reflex, which was proven to take longer to recover (Full *et al*, 2016; Hodshon & Thomas, 2018). Its recovery throughout time may be associated with all the neurorehabilitation efforts, mainly the repetitive stimulation of the pre-synaptic synapses and spared pathways through a multimodal approach (Gouveia *et al.*, 2022c).

The evolution of the SSS mean scores according to aetiology (Fig. 22) revealed that non-compressive aetiologies (ANNPE and FCEM) had a faster decrease in SSS scores. This might have happened due to the more intensive neurorehabilitation protocol these patients were subjected to from the start. Comparatively to the compressive aetiology patients, they were subjected to a faster speed, longer sessions, and more repetitions in the land treadmill locomotor training, in addition to being able to initiate underwater treadmill training right at the time of admission. Compressive aetiology patients could not follow this protocol during their early stages mainly to prevent compromising the spinal stability and risking any suture infection.

Locomotor training showed time and time again to be of utmost importance for functional recovery (Grasso *et al.*, 2004; Rossignol & Frigon, 2011; Martinez *et al.*, 2012; Côté *et al*, 2017; Hodgson *et al.*, 2017; Yu *et al.*, 2019; Gouveia *et al.*, 2022a), including in SS cases. This repetition-based exercise enhances CPG's activity responsible for the left and right alternating, rhythmic stepping movements (Rossignol & Frigon, 2011; Martins, 2015; Yu *et al.*, 2019), through firing and inhibiting the flexor and extensor muscle groups in succession (Martins *et al.*, 2021c), and, with time, it improves patients' gait and coordination (Gouveia *et al.*, 2022a). Moreover, the sensory feedback provided by the treadmill belt

as well as the water viscosity and temperature in UWTT stimulates the afferent cutaneous receptors present in the foot, which have shown to be crucial for modulating and adapting each step accordingly to the environment (Rossignol & Frigon, 2011; Côté *et al*, 2018).

The combination of LT and ES, which encourages neuroregeneration by promoting the sprouting of new peripheral circuits and the regeneration of spared ones (Zheng *et al.*, 2020), as well as aiding in slowing down the muscle atrophy through the conversion of muscle fibers type II (fast fatigable) to type I (fatigue resistant) (Sheffler & Chae, 2007; Côté *et al*, 2017), is a multimodal approach to neurorehabilitation thought to be the most effective way to regain ambulation (Ragnarsson, 2008; Martins *et al.*, 2021b) as it has shown to decrease functional recovery time (Zheng *et al.*, 2020). Additionally, the use of laser therapy prior to LT and ES may be beneficial to reduce pain and inflammation allowing a more effective neurorehabilitation session (Gross, 2014). Given these intensive neurorehabilitation efforts, 42 patients were able to reach a SSS score of zero (Table 8) during the first 72h and were, therefore, removed from the rest of the study.

This study included a total population of 249 dogs, stablishing a level of power (1-β) of approximately 0.90 and a type I error (α) of 0.01, in other words, there is a possibility of one false positive in 100 observations (Schulz & Grimes, 2005). As for the inter-rater reliability for the SSS, there was an 18% disagreement between the two raters.

The lack of statistics for the Cronbach's α for internal consistency evaluation to assess the scale's reliability are some of the limitations of the study, as well as not having faecal and urinary incontinence records. On the other hand, since the SSS does not have a gold standard scale or any other scale that it can be compared to, it was not possible to determine criterion and construct validity. Additionally, electromyographic studies as well as the possibility to adjust FES parameters through compound muscle action potential (CMAP) analysis, to decrease muscle fatigue and prevent hyperexcitability leading to spasticity could be helpful in future studies. On top of that, the development of biomarkers measurements to exclude prematurely progressive myelomalacia could be helpful.

6. Conclusion

In conclusion, more than 70% of the patients were admitted under 48h post-injury, allowing the early detection of SS signs. Even though these patients presented with higher SSS scores, they also had faster recoveries of SS, as expected from its natural progression but, also, due to the possibility of initiating a neurorehabilitation protocol sooner. Patients with non-compressive myelopathies showed a much faster recovery of SS, probably due to the difference in neurorehabilitation protocol that allowed them to be stimulated with more intensity in comparison to compressive myelopathy patients. In addition, patients without DPP had a slower recovery compared to the ones with DPP. Also, only the latter group and the ones that were able to regain DPP were able to reach ambulation.

As for the time of writing of the present dissertation, the author believes that SS has not been giving the proper importance in the veterinary and neurorehabilitation field, given its effects on the recovery of locomotion have been demonstrated, especially in dogs without DPP, as well as its influence over the time it takes to achieve ambulation again.

The implementation of an early neurorehabilitation protocol for SCI patients has already been proven important (Moore *et al.*, 2020; Olby *et al.*, 2020), however this new SSS classification can be helpful to adjust each protocol to an individual patient's needs, in particular to those with a SSS ≥ 4, by potentially serving as a checklist to decide prematurely which electrical modality to use with the aim of attaining neural reorganization, strengthening muscle tonus, focusing on neurotransmitter balance and, overall attempting to oppose the SS phenomenon. Additionally, the use of repetitive exercises, FES protocols, and the application of TESCS could be helpful to strengthen abdominal and perineal muscle tonus.

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ANNEX I – Open Field Scale

Table 9: Five Stages of the Open Field Score (Source: Olby et al., 2001)

Stages	Score	Description
Stage 1	0	No pelvic limb movement and no deep pain perception
	1	No pelvic limb movement with deep pain perception
	2	No pelvic limb movement but voluntary tail movement
Stage 2	3	Minimal non-weight-bearing protraction of the pelvic limb (movement of one joint)
	4	Non-weight-bearing protraction of the pelvic limb with more than one joint involved less than 50% of the time
	5	Non-weight-bearing protraction of the pelvic limb with more than one joint involved more than 50% of the time
Stage 3	6	Weight-bearing protraction of pelvic limb less than 10% of the time
	7	Weight-bearing protraction of pelvic limb 10% to 50% of the time
	8	Weight-bearing protraction of pelvic limb more than 50% of the time
Stage 4	9	Weight-bearing protraction 100% of the time with reduced strength of pelvic limb. Mistakes >90% time
	10	Weight-bearing protraction 100% of the time with reduced strength of pelvic limb. Mistakes 50%-90% time
	11	Weight-bearing protraction 100% of the time with reduced strength of pelvic limb. Mistakes <50% time
Stage 5	12	Ataxic pelvic limb gait with normal strength but mistakes made >50% of the time
	13	Ataxic pelvic limb gait with normal strength but mistakes made <50% of the time
	14	Normal pelvic limb gait

ANNEX II – Functional Neurorehabilitation Scale for dogs with no deep pain perception

Table 10: Functional Neurorehabilitation Scale for dogs with no deep pain perception (FNS-DPN) (Source: Martins et al., 2018)

	Description		Score
Nociception evaluation	Deep pain perception present in the digits		"+1"
	Deep pain perception present in the tail		"+1"
	Deep pain perception present in the perineum (dermatomes S3)		"+1"
	Deep pain perception present in the vulva (dermatomes S2)		"+1"
Spinal reflexes evaluation	Patellar reflex	Absent	0
	Cranial tibial reflex	Decreased	1
	Withdrawal reflex	Normal	2
		Increased	3
	Crossed extensor reflex	Absent	0
		Present	1
Muscle tone evaluation	Hypotonic extensor muscles and hypotonic flexor muscles		0
	Hypertonic extensor muscles and hypotonic flexor muscles		1
	Spasticity of the extensor and hypotonic flexor muscles with passive range of motion difficult or absent		2
	Hypertonic extensor muscles and hypotonic flexor muscles with decreased range of motion		3
	Normal muscle tone or slightly hypotonic flexor muscles		4
Gait evaluation	Paraplegia		0
	Presence of movement without deep pain perception, non-functional		1
	Presence of movement with deep pain perception, non-functional		3
	Presence of movement without deep pain perception, fuctional		2
	Presence of movement with deep pain perception, functional		4
Proprioception and locomotor coordination evaluation	Coordination between pelvic and thoracic limbs <10% of the time*; +/- knuckling		0
	Coordination between pelvic and thoracic limbs 10-25% of the time; +/- knuckling		1
	Coordination between pelvic and thoracic limbs 25-50% of the time; without knuckling		2
	Coordination between pelvic and thoracic limbs 50-75% of the time; without knuckling		3
	Coordination between pelvic and thoracic limbs >75% of the time; without knuckling		4

*time – relative to 30 steps